

nature*10 August 1978*

Law of the Sea: five years of negotiations

IN two weeks' time the United Nations Conference on the Law of the Sea will be back in session yet again; a four-week period in New York constitutes the 'resumed seventh session' in a series which now extends over almost five years. Among the main outstanding issues which are certain to get much attention in New York are the shape and form of an International Sea-Bed Authority and how to reconcile the interests of those states for whom a 200-mile Exclusive Economic Zone around their territory is unsatisfactory.

The conference has been through a difficult time these past few months, indeed there have been times when it could have collapsed altogether; it is largely as a result of these difficulties that delegates find themselves back at the negotiating table so soon after a session in Geneva in April. For at that time a major dispute flared up over the presidency of the conference by Mr Hamilton Amerasinghe. Mr Amerasinghe, of Sri Lanka, had been president for all previous sessions and his continuation in the post would have satisfied most delegations, but a new government in Sri Lanka was, for party-political reasons, in no mood to let Mr Amerasinghe stay in its delegation. Two weeks in Geneva were wasted on this matter, and when a means was found of allowing Mr Amerasinghe to stay on as president for the seventh session, many Latin American delegates staged a noisy walkout and threatened to take the question of his presidency to a session of the UN General Assembly. Latin American delegates had their own candidate for the presidency, and were probably fairly uneasy about later stages of the conference, as they have already obtained what they really wanted—legitimacy for their 200-mile limits—and did not wish to see further discussions dilute that in any way. Although the second two weeks in Geneva were quite fruitful, there was undoubtedly a feeling that the wasted time had better be made up with a 'resumed' session (which allows Mr Amerasinghe to preside yet again), but a meeting following so soon seems to have stretched the resources of countries large and small, and there is some doubt on how much can be achieved.

Not every country will be satisfied by the 200-mile exclusive economic zone. On the one hand there are those with continental shelves that extend well beyond 200 miles, and they will be looking to this session for some clarification on this matter. On the other hand there are the disadvantaged nations for whom a 200-mile zone is meaningless—these include not just landlocked nations but countries such as the Netherlands. These nations are still looking for recognition of their disadvantage, maybe in the form of some income from

the exploitation of the shelves of more advantaged nations.

The question of authority over the deep-sea bed looks, if anything, still more difficult to resolve. There is wide agreement on the need for a sea-bed authority, which will not just take a hand in exploitation of deep-sea resources but will also probably have some role in research. The problem is how big the involvement should be. Many developing countries see a sea-bed authority as the first major step they can take towards making the West take the New International Economic Order seriously. The only sea-bed resource for the foreseeable future will be manganese nodules, and large-scale exploitation of them could have a dramatic effect on the world prices of manganese, cobalt and nickel. If the sea-bed authority is really in tune with the New International Economic Order, many nations will argue, it cannot allow commercial enterprises from rich parts of the world to go picking up nodules without any attention to the economic consequences in countries which depend on mining for their survival.

What will happen to scientific research under a sea-bed authority is just as problematical. A very large proportion of the work done quite openly by universities and research institutions on the sea bed can be construed in one way or another as resource-related, even if our present understanding of that part of the world is still very rudimentary. On the other hand there is plenty of military research conducted in the deep sea, some of it concerned with the sea-bed. No nation is going to allow itself to be forced to clear its military oceanography with an international authority, but the issue is not that simple as some military authorities, most notably the US Office of Naval Research, have been enlightened and liberal in their support of general oceanography; and this sponsorship might prove an embarrassment in future.

Developing countries would do well to use the sea-bed authority as a way to boost oceanographic expertise by asking, through it, for more educational and collaborative opportunities for their scientists, just as they will doubtless do as a condition for research being conducted in their economic zone. But they must be careful not to demand too much of the authority in the form of bureaucratic regulation and control of research, or the bright future that oceanography should have will rapidly be dimmed as young scientists, discouraged by news of tedious bureaucratic interference in research take their skills elsewhere. The authority must be used to raise research capabilities of all nations, not to suppress those that are already well advanced. □



Senate slashes NASA's moon rock research...

IN a move which has sent shock waves through the US geoscience community, the Senate has decided to cut from the 1979 budget of the National Aeronautics and Space Administration all \$5.7 million requested for continuing research on rock samples brought back by the Apollo moon missions. The decision, which one lunar scientist described last week as a "bolt out of the blue", and another as a "major catastrophe for the whole international research community", was made on the recommendation of an appropriations subcommittee chaired by Senator William Proxmire, widely-known for his attacks on esoteric-sounding research.

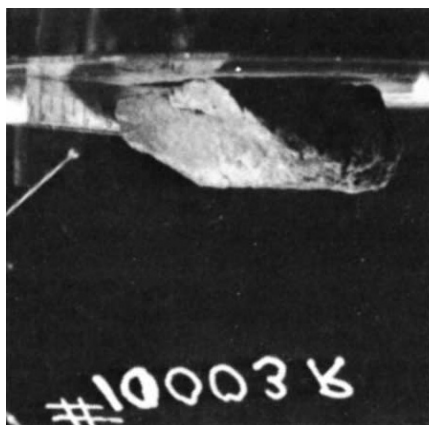
Ninety-six separate groups in universities and research institutes throughout the country are working on projects under NASA's lunar sampling analysis programme; many now face the prospect of research funds drying up from 1 February next year.

The Senate appropriations committee directed that responsibility for funding such research should be passed to the National Science Foundation. The NSF, however, is concerned that a sudden influx of high-quality applications could strain its whole earth sciences programme. The \$24.5 million budget for this programme is to be kept constant from the current year, and has not been increased by the committee to take account of the new demands.

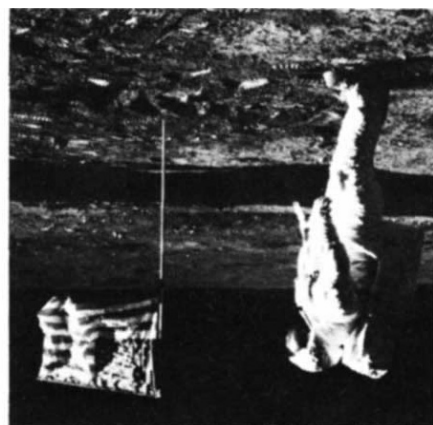
Research on the large number of lunar samples brought back by various Apollo missions since the first moon landing in 1969 has already yielded substantial information not only about the structure of the moon itself, but also about the history of other planets, for example through analysis of past radiation and particle bombardment.

Despite occasional signals from the appropriations committee in the past that basic research of this type should not be the responsibility of NASA, funds for lunar sample analysis have remained roughly constant over the past few years, and the programme has received praise for the high quality of the science conducted.

The first indication that there might be trouble with the 1979 budget request came during the subcommittee's hearings. Responding to statements about the importance of the moon rocks from Dr Robert Frosch, administrator of NASA, and Dr Noel Hinners, associate administrator for space science, Mr Proxmire quipped that if the rocks were so valuable, perhaps scientists should pay for the privilege of studying them. Mr Proxmire's subcommittee subsequently cut the full request for \$5.7 million for lunar sample analysis from the agency's 1979 budget, a move



Above: moon rock sample. Right: its collector, astronaut Aldrin. Will research now have to stop?



that, despite frantic moves by scientists to have at least part of the funds restored, was approved by the full committee at the beginning of last week.

In its report to the Senate on the bill appropriating the NASA budget, the committee said that in cutting the NASA funds, it expected the NSF to accept applications for such research. The committee added that NASA could continue to support, from other available funds, "the more promising on-going lunar sample analysis on a short-term basis while new funding mechanisms, in cooperation with NSF, are established".

Under a separate section of the bill appropriating funds to the NSF, the committee directed that proposals for lunar sample analysis "are to be evaluated competitively with other applications for funds for basic research in the geological sciences".

The lunar sample research is not the only project to have suffered at the hands of the appropriations subcommittee. The committee also deleted all funds for research into extraterrestrial life forms, a previous recipient of Mr Proxmire's "Golden Fleece" award, whose budget had already been reduced from \$2 million to \$600,000 by the House appropriations committee.

But it is the moon rock decision which is likely to have the greatest impact on the scientific community. Of particular concern to NSF is that many of the projects now funded by NASA would come under the geochemistry budget which has already been held down to under \$8 million, and can at present fund less than half the proposals it receives. An influx of new, high quality applications, could prove devastating to current programmes and new proposals alike.

"One serious consequence could be on scientific cooperation. In the past, people from different disciplines have been able to support each other when

good projects have been proposed by NASA; but the pressures to be more aggressive will become quite severe, for example possibly between those engaged in terrestrial and extra-terrestrial research", said one member of the lunar and planetary laboratory at Arizona State University.

"The outcome of this decision will be to devastate a whole area of research, not only in the US, but in the rest of the world as well", says Dr Gerry Wasserberg, professor of geology and geophysics at California Institute of Technology. "It will be a major blow to the whole space science programme, as well as to the world-wide study of extra-terrestrial materials".

Despite objections from, among others, Senator Harrison Schmitt, one of the original Apollo astronauts who described the cuts as "incredibly unbelievable", they were left standing by the Senate when it considered the appropriations on Monday. The main hope now is that at least some of the money can be restored at the conference stage, when some lively horse-trading is expected between House and Senate. The Senate appropriations committee, for example, disagreed with its House counterpart that \$30 million should be put aside from the budgets for the solar polar mission, the space telescope and the Jupiter orbiter probe to cover possible cost over-runs in the space shuttle programme.

Scientists are therefore hoping that, in return for a compromise agreement on these actions, the Senate committee will be prepared to reinstate some of the money for the lunar sample analysis, for which the full \$5.7 million request was both authorised and appropriated by the House, Senator Proxmire has said that he might be prepared to accept a figure of \$2.5 million for the programme. But even this would have a serious impact on current research.

David Dickson

... but gives \$30m back to NSF

THE US Senate agreed on Monday to restore \$30 million of the \$44 million which the House of Representatives had earlier cut from the research budget of the National Science Foundation for the fiscal year 1978.

The Senate Appropriations Committee had recommended that \$40 m be restored, in line with the sum that the Senate had already authorised. This would have brought the NSF's budget to \$927 m, only \$7 m less than the amount requested by the administration.

However, Senator William Proxmire, Chairman of the Appropriations Subcommittee responsible for the NSF budget, introduced an amendment to the Appropriations Bill during the debate on the floor of the Senate on Monday afternoon cutting 2% off the total budget of all agencies covered by the bill.

Initially the cut would have been across the board, thus reducing the NSF budget by a further \$20 m from the total recommended by the Appropriations Committee. But the amendment as finally passed by the Senate distributed the cuts between the different agencies covered by the bill in such a way that the NSF budget was

reduced by only \$10 m, leaving it up to the foundation to decide where the cuts should come.

The House, in appropriating only \$893.9 million—\$44 million less than had been requested by the administration—had made a number of cuts in the foundation's new Applied Science and Research Applications (ASRA) directorate, much of whose work, it had claimed, should be carried out by other government departments. The Senate committee, however, apparently convinced of ASRA's potential, recommended that \$67 million be appropriated for it, an increase of \$9.1 million on funds currently available for the NSF's applied research programme, and only \$6.9 million less than the total initially requested.

The committee, however, in its report on the appropriations bill, expressed concern that ASRA's emphasis upon "integrated basic research" should not become a means of diverting funds from applied to basic research, and directed the NSF "to take whatever steps are necessary to ensure that funds administered by the ASRA directorate are not channelled into pure basic research".

David Dickson

Rogers proposes commission on research ethics

REPRESENTATIVE Paul Rogers, who has been responsible for much recent US health research legislation, has introduced a bill into the House of Representatives to set up a presidential commission for the study of ethical problems in medicine and biomedical and behavioural sciences.

The commission would include health professionals, biomedical and behavioural research workers, as well as non-specialists in these areas, and would require all executive agencies to provide pertinent information requested by the chairman.

The commission would be required to conduct studies of the legal, ethical and social implications of a number of specific issues, including the allocation of federal resources for biomedical and behavioural research and health care delivery, the requirements and guidelines applicable to clinical trials, counselling and testing for genetic diseases, and developments in *in vitro*



Rogers: grappling with dilemmas.

Departments urged to support basic research

DR Frank Press, director of President Carter's Office of Science and Technology Policy, and Mr James T. McIntyre, director of the Office of Management and Budget, have written to the heads of all federal departments and agencies asking them to give 'careful consideration' to support of basic research in drawing up their budget proposals for the fiscal year 1980.

Mr McIntyre has already indicated that there is likely to be a "growth pause" in the 1980 budget, whose target deficit has recently been reduced from \$60.5 billion to \$43.5 billion due to growing concern about the effects of inflation.

In a memorandum to agency heads, the two presidential advisers say that "despite these tight constraints, we believe that it is important to re-emphasize the administration's concern for the funding of basic research".

They stress that it is the policy of the administration to assure effective support of basic or long-term research, particularly to provide a better basis for decision making or for dealing with long-term national problems.

Support for such moves has also come from the National Science Board, the policy-making body of the National

Science Foundation. In a report on *Basic Research in the Mission Agencies* which was presented to Congress by President Carter last week, the report states boldly that "basic research is useful", and emphasises that basic research has produced and continues to produce significant additions to scientific knowledge that are, or promise to be, of high potential in addressing national problems and concerns.

The report says that, in the main, mission agencies acknowledge payoffs from their investments in basic research, and points out that although there has been a 25-fold increase in current dollar terms in the amount of basic research performed in universities in the period 1953 to 1977, the performance of basic research by industry has increased by only 5 times over this period.

Dr Grover E. Murray, vice chairman of the National Science Board and chairman of the committee responsible for the report, said last week that "as a result of this review of the agencies and their activities, the board affirms its strong belief in the value of multiple support of scientific research by the federal government and in the key role of the mission agencies". □

fertilisation.

It would succeed the present National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, which has more narrowly defined responsibilities, and will go out of existence at the end of October. Six weeks ago the Senate passed a resolution sponsored by, among others, Senator Edward Kennedy which would extend the life of the present commission for a further four years.

Introducing his bill in the House last week on behalf of members of the health and environment subcommittee of the House of commerce committee, Mr Rogers said that ethical problems connected with research on human subjects still exist. The US was grappling with many very difficult ethical dilemmas in medical practice

and in the conduct of biomedical and behavioural research which went far beyond the protection of the rights of research subjects.

"It is time we began to deal with these problems directly and as expertly as possible. Our experience with the national commission has demonstrated that the concept of a working study commission is a valid one, and has provided a model which may now be applied to the study of these areas at a time when Congress and the administration is having to make crucial policy decisions."

Earlier this year, an editorial in the *New York Times* praised the work of the national commission, pointing out that its guidelines for the conduct of research on, for example, fetuses and prisoners had shaped research practices and had often been incorporated into government regulations, while fears that the commission would block valuable research had proved largely unwarranted.

However elsewhere the idea of a new commission with broadened responsibilities is likely to receive a cooler reception. Some research workers have claimed recently that research into *in vitro* fertilisation techniques, such as those used by Steptoe and Edwards leading up to the English "test-tube baby" birth, has been held up by the deliberations of a 12-person ethics advisory board set up last year by the Department of Health, Education and Welfare.

David Dickson

Geologists warn of uranium shortage

UNLESS there is a substantial increase in research and exploration efforts, demand for uranium fuel for light water nuclear reactors will soon outstrip supply, according to a report published last week by the US National Academy of Sciences.

According to the authors of the report, "if a [US] national policy decision is made to use nuclear-generated electric power as a major contribution to the total US energy supply, then at least a five-fold increase in the rates of discovery and development of reserves is necessary to meet current forecasts of future demands."

Among the measures recommended to achieve an adequate future supply of uranium is an expansion of the federally-supported research and development programme in the basic geology and geochemistry of uranium.

The report has been prepared as part of a comprehensive study of the nation's prospective energy economy during the period 1985 to 2010 currently being undertaken by the National Research Council at the request

of the Energy Research and Development Administration (now the Department of Energy).

The study is being conducted by a Committee on Nuclear and Alternative Energy Systems through an elaborate system of panels and resource groups involving more than 200 individuals. The committee's report had initially been scheduled for completion by 30 June 1977. However following difficulties in reaching a general consensus, no date for completion has yet been fixed, and it has been decided to publish in advance the reports of the various panels and resource groups, stressing that these do not necessarily reflect the view of the committee.



"Unless more money is put into research on uranium sources there won't be enough energy to power the research centre!"

The report on US uranium resources was prepared by a subpanel chaired by Dr Leon T. Silver, professor of geological and planetary sciences and the California Institute of Technology. The subpanel says that domestic uranium production has been between 12,000 and 13,000 tons of U_3O_8 for the past five years, and is estimated to reach between 17,000 and 19,000 tons by 1980, and between 22,000 and 26,000 tons by 1985. It claims that this will fall significantly short of the Department of Energy's forecasts of demand and production, and of its contracts for enrichment, if constraints on exploration and development are unchanged.

The subpanel also says that foreign exploration and demand are growing at comparable rates, and that the US will have to compete vigorously in order to obtain significant supplies of uranium abroad. The subpanel adds that the US "will have no particular exploration advantages unless it develops superior science and technology by a programme of research at home".

Current federal programmes of geological, geochemical and geophysical research relevant to uranium exploration are, it says, in general limited in concept and magnitude, inadequate in funding direction and co-ordination, and do not utilise the best talents and technical capabilities available in the US research community. □

Select Committee recommends itself for fast breeder inquiry

THE UK Select Committee on Science and Technology, clearing its cupboards before a possible general election, has recommended that it should hold a public inquiry into the future of Britain's Fast Breeder Reactor (FBR) programme. The inquiry should be completed within a single session of Parliament, preferably the next one.

In 1976 the Select Committee was told by the UKAEA that a decision to construct a commercial demonstration fast reactor (CDFR) must be reached within two years; but since then there has been a considerable change of atmosphere, with no firm proposal forthcoming from either the UKAEA or the Government. During the Wind-scale Inquiry into the reprocessing of nuclear fuel, Energy Secretary Tony Benn pledged an inquiry into FBRs without specifying what form or terms of reference it should adopt. The Select Committee now hope that their own inquiry would help to precipitate some decisions.

The Select Committee aims to inquire into the wider issues raised by FBRs, rather than study any particular proposal, such as the CDFR. Mr Arthur Palmer, the Committee chairman, said last week that the Committee hoped to avoid the ground already covered "by the numerous comprehensive and exhaustive enquiries into FBR policy options and technology which have already been made in North America and Western Europe". Rather it should concentrate on the implications of the previous work, whether general to FBR technology, or peculiar to its development in Britain.

Certainly there must be a further inquiry as soon as definite proposals for the CDFR are put forward; several Acts of Parliament allow for planning inquiries, depending on the exact nature of the proposal.

The details of the Select Committee's own inquiry will not be settled until the next Parliamentary session; but by that time there may be a new Government, or the responsibility may have fallen to a new Select Committee on Energy. The key procedural problems will be to decide whether interested parties can cross-examine expert witnesses, and to determine exactly what range of subjects will be held to be relevant. Until these problems are resolved, the proponents of FBRs will remain suspicious that the inquiry will merely duplicate others, and the FBR's opponents will fear that the whole exercise is designed to rubber-stamp the fast breeder programme.

Peter Scott

China wants 800,000 scientists by 1985

CHINA would like to send 200 students a year to the UK, as part of a programme to produce 800,000 scientists by 1985, a Royal Society delegation was told in China last week. The delegation met Vice-Premier Fang-Yi, who has responsibility for China's science policy, and other leading figures. To help meet its 800,000 target China hopes to send some 10,000 of its scientists and students to other countries. To do that China "will have to send them to several scientifically advanced countries", the leader of the delegation, Royal Society Foreign Secretary Dr M. G. P. Stoker, said on his return.

China will probably send most to the US, where the Presidential Science Adviser, Dr Frank Press, has expressed his willingness to accept them. Others will come to the UK, and to France, and some to Germany. China may favour the US and the UK, because the country appears to have chosen English as its second language. But France has signed a major scientific agreement with China emphasising technology, and in Germany the Max Planck Institutes are willing to take students.

China's programme is "very ambitious", said Dr Stoker. But under the gang of four the country had been scientifically "ten years in the wilderness". The scientists the Royal Society delegation met were mostly over 35.

The Academia Sinica, which hosted the delegation, strongly emphasised China's needs in basic science. The Academy is responsible for 80 research institutes and three universities, and is "not the body primarily concerned" with the 'eight topics' developed at China's recent national conference on science and technology. "Our exchange programme is directed at all the basic sciences", said Dr Stoker.

Hao Pin, Head of the Foreign Relations Department of the Academy, has agreed to a major expansion of

collaboration with the Royal Society, to be ratified by a formal agreement after approval by the Society and the Academy. Apart from the 200 students, China wishes to send small delegations of senior research workers to visit one or two centres in the UK. Exchanging costs, the Royal Society hopes to send up to 20 senior scientists to lecture in China; but the Academy may increase the number of exchanges at its own cost.

The up to 200 students per year—100 at post-graduate and 100 at post-doctoral level—that the Academy wishes to send to the UK will be paid for by the Academy, with the Royal Society responsible for placing them. The students will stay for the usual periods: three years for post-graduates and two for post-doctorals, but none are expected to take degrees. Dr Stoker is concerned about language difficulties and the academic standards of the visitors—"I stressed that their standards must be very high".

China studies education in foreign countries

FORTY academics and state officials last month attended China's first Seminar to Study Education in Foreign Countries. In this they followed Mao's call to the Chinese people, reiterated in many of his policy speeches by Chairman Hua Kuo-Feng, that they should be good at absorbing whatever is valuable in things foreign, and turn it to their own use by combining it with their own inventiveness. Speaking at the Seminar, Vice-Minister of Education Kao Yi, said: "the starting point of our discussion is, basing ourselves on the realities of our revolution in education, to learn and put to use the strong points of other countries". Changing realities mean, one thinks, changing criteria on what are the strong points.

Among the papers read at the

There will also be no small problem in placing these numbers particularly if they all want to go to the most prestigious laboratories. "We will have to spread them around a bit". The Royal Society may also have to take on extra staff to cope with the administration; or it may approach the British Council to help share the load. The formal agreement, Dr Stoker hopes, will be ready for the visit of a high level Chinese delegation in October led by a Vice President of the Academia Sinica, Hu K'e-Shih—or for next year, when the President of the Royal Society, Lord Todd, will be visiting China.

A separate intergovernmental agreement is also anticipated following Secretary of State Shirley Williams' visit to China recently, during which the UK and China reached an understanding in principle to exchange scientists and students. These exchanges would be extra to those planned by the Royal Society.

Robert Walgate

Seminar was one on "the Evolution of Primary and Secondary Schools in Post-War England". And there were discussions on the division of secondary school education into arts and science streams. A decision was made to bring forward the publication of two reports: "Outline of Education in Foreign Countries" and "Introduction to the World's Famous Universities".

T. B. Tang

COMING SOON: Joseph Needham, the Cambridge historian of Chinese science and technology, visited China recently with Nature support to learn first hand of China's new enthusiasm for science. His three articles on the country's science policy, 1978, will appear in Nature shortly.

Conference time signals protests

As the summer conference season gets under way, many western scientists, shocked at the sentencing of dissidents Orlov and Shcharanskii, have sought their own forms of protest against the action of the Soviet authorities.

At the International Luminescence Conference in Paris last month (the first international physics meeting since the Orlov trial), a "round table" and exhibition in defence of the Russian dissident was held. The protest was deliberately low key, and accordingly,

the Soviet delegation did not feel itself obliged to withdraw from the conference as they might have done had the protest been more vociferous.

This month's meeting of the Fourteenth International Congress of Genetics in Moscow poses an even greater challenge to participants. Although none of the defendants in the recent trials was a biologist, in the Soviet Union there has always been a strong emotional link between physics and geneticists, dating back to the support given by the physicists to the "underground" geneticists during the days of Lysenkoism.

Moreover, a letter has just reached Amnesty International from Ivan

Kovalev, biologist and human-rights activist, now halfway through a seven-year sentence. The letter, which includes a detailed description of camp conditions, calls upon western participants in the conference to express their support for Kovalev "by whatever means they consider appropriate".

Other current protests aim at preventing imprisonments—notably a petition from US and British scientists, including 11 Fellows of the Royal Society and two British Nobel Prize-winners, in defence of Grigori Rosen-shtein, a Moscow cyberneticist *refusnik*, who is threatened with a trial for "parasitism" (being without visible means of support).

Vera Rich

Dioxin source is 'safe'

IN its eighth pronouncement since 1970 on the herbicide 2,4,5-trichlorophenoxy acetic acid the UK Ministry of Agriculture, Fisheries and Food (MAFF) has recently stated that it finds 2,4,5-T to be safe if used in the "recommended way for the recommended purposes". In Australia the National Health and Medical Research Council is of the same opinion after considering recent allegations that the herbicide poses a public health problem.

Concern centres around contamination of 2,4,5-T with the toxic by-product 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Recent reports suggest that traces of dioxin are produced when plants sprayed with 2,4,5-T are subsequently burned. In the opinion of the British and Australian committees, however, this does not create enough of an additional health risk to proscribe use of the herbicide. The MAFF committee is to prepare and publish a reference document on the subject.

The Swedish Natural Science Research Council, in collaboration with the Swedish Academy of Sciences, has just published such a document, which has considerable value as a reference source on the chemistry and biological properties of the compounds it discusses.

In supporting continued use of 2,4,5-T, the Swedish report makes several provisos. It considers that the stability and bioaccumulation of dioxin should be studied under normal field conditions; that manufacturing processes for production of the herbicide could be improved, thus reducing the potential dioxin contamination of the environment in the event of an accident; and that other synthetic processes that eliminate dioxin formation should be investigated. It also recommends studies of the mutagenic properties of the herbicides, and epidemiological investigations of human groups exposed to 2,4,5-T, 2,4,5-trichlorophenoxy propionic acid, and their dioxin contaminants.

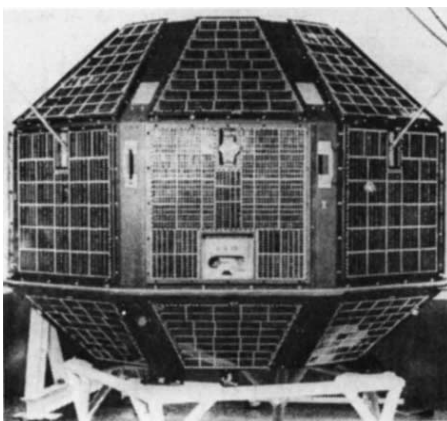
A large-scale epidemiological study of 2,4,5-T and its relation to infant malformation rate is currently in progress in New Zealand. The results of this study may provide evidence to assist the United States Veterans Administration which is currently considering claims from veterans of the Vietnam war that exposure to 2,4,5-T during herbicide spraying operations in Indo-China has affected their health and, in some cases, that their children were born malformed.

Alastair Hay

India prepares to launch Earth resources satellite

INDIA will launch its second satellite at the end of this year—the Satellite for Earth Observation (SEO). This satellite should fulfil one of India's main objectives in space: the "management of natural resources through remote sensing technology"; and it should also satisfy national pride, in that it uses 85% indigenous technology.

SEO is designed to operate for a year, and should produce data on agriculture, forestry, hydrology, and oceanography. It will observe the earth with two TV cameras with a ground resolution of 1 km, and two microwave radiometers. It will be launched by a Soviet Intercosmos rocket into an orbit of altitude 525 km and inclination 51° from the Sriharikota range (SHAR), Andhra Pradesh.



Aryabhata, India's first satellite launched in 1975.

India began its space effort when the Thumba Equatorial Rocket Launching Station, near Trivandrum, an international facility for launching rockets with scientific payloads, became operational in 1963. The station is now a part of Vikram Sarabhai Space Center (VSSC), whose primary aim is to build and launch indigenous rockets complete with payloads. Facilities for monitoring India's first satellite Aryabhata were completed in 1975 at the Sriharikota range. At the range rocket launching facilities are currently nearing completion. A new satellite tracking and ranging station, equipped with a stellar camera and a ranging laser, was also established last year at Kavulur, Tamil Nadu.

India first entered space in April, 1975, when an Intercosmos rocket launched 'Aryabhata'—a 360 kg satellite carrying a payload to study aeronomy, solar neutron flux and X-ray astronomy. But only five days after launch, the payload was switched off

due to a malfunction. The satellite itself, however, went on to exceed its expected six months' life time. Soon after that launch, another agreement was signed between the Indian Space Research Organisation (ISRO) and the USSR Academy of Sciences to launch SEO in 1978.

In the 1980s India plans to have its own INSAT (Indian National Satellites) system for domestic purposes. The biggest effort in this area so far has been the Satellite Instructional Television Experiment (SITE) programme. From August 1975 to July 1976, clusters of 2,400 villages in six states were shown TV broadcasts via NASA's butterfly ATS-6 satellite. The rural populace was provided with programmes on new food production techniques, health and sanitation as well as with educational and entertainment programmes.

The current two year Satellite Telecommunications Experiment Project which began in June 1977 has a similar aim. The Franco-German "Symphonie" satellite is linked with three static earth stations at Delhi, Ahmedabad and Madras, and two mobile ones. The latter may be used to establish communications in remote areas or during an emergency.

India is also building at VSSC a four stage solid propellant rocket, Satellite Launcher Vehicle-3 (SLV-3), which should be ready by the end of the year. It is due to place a 40kg indigenous Rohini satellite into a near earth elliptical orbit during 1979. The satellite will monitor the fourth stage of the SLV-3. The aim is to develop a launcher suitable for heavier remote sensing satellites.

The ISRO has also booked a berth abroad the European Space Agency's launcher Ariane to launch APPLE—India's indigenous 616 kg experimental comsat—in May 1980. Skills acquired in building and operating this geosynchronous satellite will be of use in the multi-purpose INSAT system. However, because of "parking problems" in space over the Indian Ocean, India is eager to have its own comsat as early as possible. Already a longitude of 79° East has been reserved for it. The US Ford Aerospace Corporation, has recently been given the contract to build INSAT, which will be placed in orbit by either a Titan rocket or the US Space Shuttle. 4.5 billion Rupees has been allotted to India's Space Programme over the next five years—more than the total investment made in the last fifteen years.

Dilip M. Salwi

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Mark Edwards: IPPF

Mark Edwards: Earthscan

Traditional bullock carts: just right for local conditions. Modernising them could be beyond village craftsmen.

Beware of imported technology, says Indian group

IMPORTED technology benefits the rich but not the poor, believes one group of scientists in India. This is ASTRA, the Cell for the Application of Science and Technology to Rural Areas, at the Indian Institute of Science (IIS) in Bangalore. And ASTRA is determined to provide alternatives.

A group at the Institute started the ball rolling four years ago with a series of seminars on rural problems. These were intended to get members in various departments to start practical projects on these problems. The underlying philosophy was that most imported technology will tend to benefit the urban population or the relatively rich villagers and may even worsen the lot of the rural poor. Given this, the group argued, developments are needed which require low capital investment and maintain or increase the level of employment. Since they have to be accepted by villagers developments must also operate within the framework of the Indian village.

The group formed itself into ASTRA. The first problem it faced was that the villages are already very developed systems with a rigid social structure and with virtually complete, if inefficient, usage of all the available resources except manpower. Any dramatic developments when introduced into this framework are likely to unbalance the whole society and work to the detriment of the poorest people. So ASTRA had to aim to work within the system producing a series of technical perturbations which can be absorbed into village life without any social upheaval. To promote this ASTRA has an extension centre in a village about 100 km from Bangalore, close enough that it is easy to visit but far enough that the academic visitors will have to spend the night there and so will be encouraged to see their work in its context.

One project which has attracted a

lot of attention is a study of bullock carts. There are about 12 million of these single axle carts in India, worth about £100 each. ASTRA engineers have analysed the vertical and horizontal forces on the bullock as a function of wheel diameter, ground slope and friction. They concluded that the usual unsprung cart with large wooden wheels, is already excellent for the transport of light loads over rough grounds. ASTRA is studying the possible advantages of pneumatic tyres and ball bearings, though these have the disadvantage that they are beyond the scope of the traditional wheelwright. Measurements on carts mounted with load cells are being made to check the calculated results. Recently a shortage of teak needed to construct the wheels has led to studies of other woods which might have the correct combination of mechanical properties and dimensional stability.

Another project illustrating the main themes of ASTRA concerns the use of a simple press to make mud blocks for house walls at lower cost than blocks or bricks made by hand. An analysis has been made of stress distributions in a mud-walled house and of the strength properties of the blocks. The major problem with mud walls is weather resistance and studies are being made of improvements with small additions of cement. Using this system a single storey laboratory has been built to house a project on methane production from waste.

Many people feel that India should be endeavouring to eliminate bullock carts rather than to improve them and thus consider ASTRA's efforts to be misguided. It is also tempting to think that rural life needs major social changes rather than minor technological ones but so far the village has proved to be a very stable, conservative society. Since ASTRA's approach is relatively cheap and the adverse con-

sequences of their innovations should be minor, they really only have to prove that they can produce effective changes. This means producing modifications to existing methods which are within their guidelines of being labour-intensive, fuel-saving, and acceptable to the villagers. An ASTRA report on an exhibition of low cost houses designed by various research groups makes the point very strongly that an acceptable result is unlikely unless close links are maintained with a rural area. Faults in these houses included use of industrial materials such as plywood, poor thermal insulation and designs which left no space for stabling animals under the same roof.

A further point which has to be proven is that such "alternative technology" research can be usefully housed within universities. ASTRA is essentially a loose grouping of faculty whose primary allegiance is to their academic departments. It is held together by a convenor, currently Dr Amulia K. N. Reddy, who must maintain a feeling of unity within the group and act as the representative for the group as a whole to the outside world. For such a group to hold together it is necessary that the research can be seen to involve application of advanced scientific and engineering methods on a par with those in other types of research. The examples given above involve aspects of civil and mechanical engineering and materials science and other projects clearly involve chemistry and chemical engineering. A demonstration that these projects do have strong roots in the pure sciences would go a long way towards supporting the idea that pure scientific research should be maintained in underdeveloped countries as a resource for applied research and to justifying European training in pure research of students from these countries.

Paul Calvert

correspondence

Australian conservation

SIR,—Kenneth Mellanby's impressions of nature conservation in Australia (4 May, page 7) are based on the short visit he made there early this year. For readers unfamiliar with Australia, his account could be misleading.

Mellanby, together with the Association for Regional Parks and Countryside Commissions of Australia, ARPCCA, the organisation which promoted his visit, question the wisdom of establishing large national parks in much of Australia, preferring instead the smaller, man-modified nature reserve and countryside park of the British type. National parks and countryside parks are represented more as competing alternatives than as different but complementary aspects of nature conservation. Application of the British approach in Australia would make it difficult if not impossible to reserve extensive tracts of land that either still exist in, or can be restored to near the condition which existed at the time of European occupation; this possibility exists in Australia but in few other places. The importance of such reservations for scientific study and reference, maintenance of large gene pools, education and recreation is widely recognised.

All states and territories of Australia have made rapid progress in recent years through their national parks and wildlife services or equivalent conservation agencies. In 1968 approximately 1.1% of Australia was in national parks and similar reserves, 2.1% in 1972, and 3.1% in June 1977. The progressive achievement of the goal of an ecologically representative system of reserves is also protecting most species of the Australian flora and fauna, without the need for the "sophisticated management" Mellanby considers necessary "in Britain and the developed parts of Europe".

We recognise, as does Mellanby, that there may be management problems such as the persistence of blackberry thickets associated with the regeneration of modified ecosystems to near-natural conditions. But it should be pointed out that these problems exist only in limited parts of a few national parks, and that they are not sufficient reason for omitting or excising potentially important areas from the reserve system. The national parks and wildlife agencies are now committing larger resources to problems such as weeds and feral animals. We also acknowledge problems of fire and 'fire hazard' in some national parks.

Much of the increasing ecological research in national parks relates to fire, whilst at the management level an array of measures, ranging from frequent control burning to complete protection, may be applied to different ecosystems within the same park. A long term objective for many extensive areas of the parks is that they should become self-managing. For this to occur the parks must be large enough to accommodate the effects of major disturbances such as wildfire and drought and to provide the

total habitat requirements of most of the native species of flora and fauna. Many of the ecological successions occurring within national parks and reserves in Australia have time spans of several decades, others of centuries.

We have no argument with Mellanby on the importance of and need for countryside parks and similar reserves, in which traditional agricultural practices are a major component of the ecosystem and are an important tool in maintaining it and changing it to secure specific objectives in nature conservation as well as economic production. More countryside parks are needed in Australia; but they should be developed within the extensive lands already given over to various types of economic production, not in the remaining areas of near-natural land including the national parks.

Mellanby does not explain that most national parks and wildlife agencies now make provision for the equivalent of countryside parks as wildlife refuges, game reserves, or similar reserves in which economic types of primary production are integrated with wildlife management, according to a prescribed management plan drawn up jointly by the wildlife agency and the landholder. There are also provisions under town and country planning legislation for land zoning so that important natural features can be retained within the framework of economic land uses. The main difficulties at present are not so much associated with government policy as with the reluctance among private landholders to commit themselves to forms of land use which impose restraints and obligations on how they might use parts of their land. In Australia, there is need both for more countryside parks within existing farmlands, and for more national parks in some of the major natural ecosystems not at present protected.

It seems a pity if some who see the need for a balanced land use including nature conservation should, because of differences they attach to various components in that balance, make the task more difficult for others of us in Australia who are working to satisfy this need.

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Real advances in neurotoxicity have been made

SIR,—In Alastair Hay's article 'Neurotoxins may go unrecognised' (20 July, page 206) the emphasis on the recent findings of Schaumburg and Spencer is very pertinent because they have shown for the first time the multi-focal nature of the primary changes in nerves subjected to the neurotoxins acrylamide and hexane. However, I am very surprised and disappointed that in the generalisation from the studies on acrylamide and hexane and the discussion on the development of new

tests no mention is made of advances in knowledge of the delayed neuropathy produced by organophosphorus compounds.

Due to work by many scientists, but especially by my colleague Dr M. K. Johnson, the primary chemical lesion is now known. The US Environmental Protection Agency is well aware of these advances and organised a meeting in Washington in 1976 to discuss the relevance of these findings. It is now possible to measure the activity of a particular esterase as a much more quantitative measure of this type of neurotoxic potential. I understand that the EPA may now request such additional information for their pesticide clearance system.

While we would all agree that more research work is required in the area of neurotoxicity (indeed this unit is currently studying the different mechanisms of toxicity of six classes of chemicals at both the pathological and biochemical level) it is doing a disservice to the science of toxicology not to point out when real advances have been made. Definitive reviews are available in *Archives in Toxicology*, and *Critical Reviews in Toxicology*.

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Masai and heart disease

SIR,—In his letter on the lipid hypothesis (8 June, page 422), Turner states that "as regards the Masai, the relevant fact is that they have very low serum cholesterol levels which would account for their freedom from CHD". No-one would dispute the importance of low serum cholesterol levels in determining the incidence of heart disease, but surely the question of most relevance to the hypothesis under consideration is *why* the Masai should exhibit such low values, given the cholesterol-rich nature of their staple diet of milk and ox blood. Lutz (3 November, page 8) has suggested that a low carbohydrate content in the diet may be responsible.

In a study comparing samples of tribal Masai with Masai who had been living in large cities such as Nairobi for ten years or more (Day *et al.* *Atherosclerosis*, 23, 357–361, 1976) we found a significant increase in serum cholesterol levels in the urbanised sample—despite the fact that the cholesterol content of their food had diminished. One possible explanation is the substitution of carbohydrate for animal fats in their diet. Other possible causes are an increase in stress associated with urban life, or a decrease in level of activity.

We do not claim that modification of diet has no value in lowering serum cholesterol levels, but we would suggest that the facts are by no means as clear-cut or 'formidable' as Turner would have us believe.

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news and views

Adenovirus up to date

from a Correspondent

THE shimmer of hyperbole which surrounds the titles of several recent papers on adenoviruses dramatises the radical nature of the information they contain. Particularly exciting, of course, has been the discovery of spliced mRNAs in which many adenovirologists take parental pride, and it was therefore not surprising that transcription and splicing were the source of a good deal of the discussion and speculation which went on at Örenäs, Sweden during the biennial EMBO meeting on the Molecular Biology of Adenoviruses.

Transcription and splicing

At early times during lytic infection when the viral genome seems to be arranged in a nucleosome-like configuration (Tate, Uppsala), transcription occurs from at least four promoters, two on each viral DNA strand, to yield primary transcripts each of which is complementary to one of the four main blocks of early genes. As judged by electron microscopy (H. Westphal, NIH) the majority of these RNA molecules which accumulate in the nucleus are colinear with the viral DNA and for the most part their 5' and 3' ends are identical to those found in mature, cytoplasmic early RNAs. However pulse-label experiments (J. Darnell, Rockefeller University) indicate that the primary transcripts derived from at least two of the early gene sets extend for a short distance beyond the DNA sequence which marks the 3' end of the mature mRNA. A similar continuation of RNA polymerase past the site(s) of addition of poly(A) to late mRNAs has already been described, but these results provide the first evidence for nuclear modification of the 3' end of early primary transcripts.

The four major initial transcripts are then processed by splicing into a flock of 18–20 species of early mRNAs, each of which presumably specifies a separate protein. Although the rules for early splicing remain indistinct, the pattern of events is simple enough. In all cases so far studied, splicing involves removal from the body of the RNA, one or two tracts of nucleotides

which contain termination codons in all three phases. Why all early regions so far sequenced should contain termination codons and why they should occur in clusters are unknown, but the fact that similar situations occur in the early genes of both polyoma virus and SV40 suggests that the phenomenon may be of general importance. In a tautological sense, then, the purpose of splicing early mRNAs is to remove chain terminators. True late genes by contrast, are free of terminators (the only possible exception being the gene for the non-structural viral protein, 100 K) (Galibert, Paris). Most adenoviral late genes are located on the *r* strand of the viral DNA whose transcription at late times during infection is dominated by a major promoter which maps at position 16.5. Transcription begins at this point and proceeds rightwards along the entire length of the viral genome. Before the primary transcript is completed, a nick which serves as the site of addition of poly(A) is introduced at one or other of five major locations, thereby dividing the transcript of the *r* strand into five separate domains (Darnell). The addition of poly(A) seems to precede a complex series of splicing events which eventually leads to the production of several species of late mRNA from each of the separate domains. In every case the poly(A) tracts and the sequences immediately proximal to them are conserved through the splicing process (E. Ziff, Rockefeller University), so that the mRNAs derived from any one domain contain coterminal 3' ends (L. Chow, Cold Spring Harbor; S. Berget, MIT). Thus it seems that the abundance of different late mRNAs is determined primarily by poly(A) addition (Darnell).

However, this cannot be the only factor because of evidence which indicates that the 5' end of late genes also has a role. The duplication mutant, Ad2⁺ND1 dp2 contains two insertions of SV40 DNA separated by 390 base pairs of adenoviral DNA. Although both insertions are transcribed into hybrid mRNAs consisting of adenoviral sequences at their 5' and SV40 sequences at their 3' ends, each

of them is controlled in a different way. The left hand insertion falls under the influence of an early promoter and its transcripts are never found in large quantity. The right hand insertion, however, is placed quite firmly under late control and is present in high abundance at late times during infection. Because the 3' ends of both transcripts are apparently identical the difference in abundance between them must have some other cause. The 390 nucleotides which separate the two insertions include the sequences immediately preceding the 5' end of the structural gene for fibre (S. Zain, Cold Spring Harbor) and it would therefore seem likely that the hybrid mRNA derived from the second insertion is treated as a late mRNA by virtue of the presence of these sequences.

It seems very probable that splicing of late mRNAs occurs in multiple steps, because viral transcripts have been isolated from both nucleus and cytoplasm of infected cells whose structures are those expected of intermediates (Chow; Berget). At least formally these intermediates can be divided into two independent groups—those concerned with the generation of the tripartite leader sequence and those concerned with connecting the coding sequence of the RNA to the leaders. The dividing line between these two processes occurs near map position 28. The tripartite leader sequences seem to be formed by a cut-and-patch mechanism in which small segments of RNA are removed progressively from the sequences which lie between the promoter and position 28 until only the tripartite leader (derived from positions 16.7, 19.7 and 26.7) remains. Connecting the leaders to the coding sequences involves bringing together the 5' ends of the genes which lie between position 28 and the transcript destined to be preserved (Chow; Berget). Presumably this process, and the subsequent removal of the cluster of 5' ends occurs by some sort of intramolecular recombination between the 5' ends themselves, and is perhaps mediated by some sort of effector molecule (either RNA or protein).

Splicing of the transcripts of most

SINCE the initial discovery of bizarre happenings during eukaryotic mRNA biosynthesis there has been considerable discussion of the mechanism by which intervening sequences are removed. The bulk of circumstantial evidence pointed to a mechanism which operates on an initial RNA transcript containing a linear representation of the genome. By folding this transcript into an appropriate conformation, it was imagined that the sequences to be joined could be brought together and a concerted cleavage-ligation reaction accomplished. Among the many genes which are now known to contain intervening sequences, the yeast tRNAs for tyrosine and phenylalanine are unusual in that the extra sequences which occur within their anticodon loops are rather small—14 and 18 nucleotides long, respectively. Their existence was deduced by direct DNA sequence analysis of cloned segments of yeast DNA encoding these tRNA genes (Goodman *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 5453; 1977; Valenzuela *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 190; 1978). Two recent papers (Knapp *et al. Cell* **14**, 221; 1978; O'Farrell *et al. Nature* **274**, xxx; 1978) now report that corresponding precursors have been isolated which are longer than the mature tRNAs by 14 and 18 nucleotides. Direct RNA sequence analysis has shown that they contain the mature 5' and 3' terminal sequences and that the extra nucleotides correspond to the intervening sequence present in the genome. Their isolation (Hopper *et al. Cell* **14**, 211; 1978) was

Intervening sequences excised *in vitro*

from Richard J. Roberts

possible by virtue of a mutant strain of yeast, *ts136* (Hutchinson *et al. J. Bact.* **99**, 807; 1969) which is defective in the transport of RNA from the nucleus to the cytoplasm, although the precise lesion is unknown. Now that these specific precursors are available, it is possible to assay for an enzyme activity able to remove the intervening sequences, and indeed such an activity has been detected in crude extracts of yeast cells (Knapp *et al.*, Valenzuela *et al. op. cit.*). Preliminary evidence suggests that it requires only Mg^{2+} and ATP in order to function but more detailed parameters, as well as the number of activities, must await further purification. It is present in the ribosomal wash fraction and if this reflects its intracellular location the accumulation of precursors would be explained by the failure of the mutant strain to transport them out of the nucleus. This might in turn suggest that this enzyme is quite distinct from the enzymes required to process nuclear RNAs into mRNAs since the latter must surely reside within the nucleus or its membrane. The remarkably short intervening sequences found in the tRNA genes also serve to differentiate them, and hence possibly the mechanisms for their removal,

from their mRNA counterparts.

While the fine details of the mechanism must await the further purification of the splicing enzyme, it should already be possible to determine whether the intervening sequences are removed as a linear or a circular oligonucleotide. This distinction could be important, for the latter finding would imply a reciprocal mechanism. Splicing would then lead not only to the joining of non-contiguous sequences to form functional mRNAs, but also to the joining of non-contiguous sequences from the intervening regions. The resulting RNA sequences might have interesting properties, both as a result of their novel joints and of their circular nature. The only circular RNA species known at present is the genome of the potato spindle tuber viroid (Gross *et al. Nature* **237**, 203; 1978) which certainly invites intense speculation as to its mode of infection. No gene products have been identified either *in vivo* or *in vitro* and since it lacks both a functional 5'-terminus and an AUG codon, this is perhaps not too surprising. Nevertheless, it plays such havoc within infected cells that it seems reasonable to believe that it may act on some regulatory system. Could it be that the RNA itself is directly responsible? If so, then RNA may have a crucial role in gene control—a theoretical possibility that may provide some experimental ideas about the role of intervening sequences and RNA splicing. □

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late genes is both rapid and precise. However, fibre mRNA seems to be an exception: it often contains super-numerary leader sequences derived from the early genes which lie just distal to the fibre structural gene (Chow). The presence or absence of these extra leaders does not seem to affect the translation of mRNAs (at least in cell-free systems) because mRNAs containing four leaders work as well *in vitro* as mRNAs with only three (A. Dunn, Cold Spring Harbor). However, there are indications that sequences contained within the conventional tripartite leaders may be necessary for efficient translation of late mRNAs. First, the activity of several late mRNAs in cell free systems is partially suppressed by the presence of DNA sequences complementary to the leaders (Paterson, NIH). Furthermore the first leader contains a sequence which, being complementary to the sequence at the 3' end of 18S

ribosomal RNA may be involved in a Shine/Dalgarno fashion in binding of mRNAs to 40S ribosomal subunits (Ziff).

Nomenclature

In addition to scientific matters, problems of nomenclature were also discussed at the conference. The participants considered it inadvisable at present to subscribe to the use of new terms that attempt to label those parts of a primary RNA transcript that succeed in reaching the cytoplasm compared with those parts which are presumably destroyed in the nucleus. This decision rests on the fact that from both early and late adenovirus transcription units—probably the same is true for papovaviruses, retroviruses and eventually may be shown for cellular transcription units as well—multiple spliced mRNA arrangements are possible. Thus what is conserved in one primary RNA transcript may be

discarded in another and *vice versa*. The terms 'intron' and 'exon' which have been suggested to describe regions of DNA from which discarded or conserved RNA sequences are transcribed were thought particularly inadvisable as they name regions of DNA whereas the molecules on which the choice for conservation or destruction is exercised are primary RNA transcripts. Perhaps the simplest course at the moment is to refer to regions of a primary transcript that never reach the cytoplasm as 'spacers' (in line with common practice for pre-rRNA) or 'intervening sequences' and to regions that may reach the cytoplasm as 'mRNA sequences.'

Splicing mechanism

Clearly, further progress in elucidating the mechanism of splicing of late adenoviral RNA will depend on the isolation of the enzyme(s) responsible as well as the determination of the

RNA sequences at the exact splice points. There were no reports of success with the first of these aims, but two groups (Uppsala and Cold Spring Harbor) presented preliminary analyses of the sequences which lie upstream from the coding regions of both hexon and fibre mRNAs. Although exact localisation of the splice points must await the sequencing of the appropriate regions of the viral DNA there is every reason to believe that the delay will not be too great. Between them, various groups (Ziff; Galibert; Maat, Leiden; J. Sussenbach, Utrecht; Akusjärvi, Uppsala; Zain) have sequenced between 25% and 30% of the total adenoviral genome, including many of the regions of greatest interest. The major segment which still remains untouched is that mapping between positions 17.5 and 28. In view of their obvious importance for splicing, it would be surprising if these sequences were not determined fairly quickly.

Proteins

If progress at the nucleotide level is satisfactory, the situation with early proteins is less comfortable. There is still some confusion on the exact number of early proteins or their molecular weights. Only three of a possible total of 20 early proteins have been purified (A. Levine, Princeton University; T. Persson, Uppsala) and only one of these (the 72 K DNA binding protein) has been shown to carry biological activity (M. Horwitz, Albert Einstein College of Medicine, New York). Furthermore the origins of two of the most important virus-specific proteins—T antigen, and the protein attached to the 5' ends of the viral DNA—remain obscure. It is clear that the terminal protein which is found covalently linked to the 5' termini of adenoviral DNA is also attached to the ends of replicating DNA and it seems likely that it is involved in initiation of viral DNA synthesis (G. Pearson, Oregon; J. Sussenbach, Utrecht; D. Rekosh, NIMR, London). However definitive peptide maps are not available and the protein has not been assigned to any viral gene.

At least two virus-coded proteins (15 K and ~ 48 K) specified by the region of the viral DNA responsible for transformation are precipitated by antisera that react with T antigen (Levine; Lupker, Leiden; Wold, University of Washington, St. Louis). There would seem to be no problem in accommodating the gene for the 15K protein within the DNA sequence of the left hand of the viral genome. However, the largest tract of contiguous sequence in the left half of this region that is free of terminators in any one phase could code for a protein

of molecular weight of only 28 K (Maat). Thus the mRNA for 48 K is either multiply spliced (for which there is little experimental support) or the protein has such an aberrant structure that it migrates through SDS-polyacrylamide gels in a highly anomalous fashion (for which there is little precedent). In any case the function of these T antigen related proteins remains unknown.

The patchwork arrangement of the adenoviral genome is completed by two genes whose products accumulate late in the infectious cycle although their templates lie outside the 'late' gene unit which is transcribed rightwards from the promoter at position 16.5. Convincing evidence was presented that one of these, located within an 'early' region and specifying polypeptide IX, is expressed in the absence of viral DNA replication (Persson) and is therefore regulated in a different fashion from the canonical 'late' genes. The mRNA for polypeptide IVa₂, a protein which

seems to function during maturation of the virus particle (Mathisens, Uppsala), also makes an earlier appearance than most 'late' mRNAs (Chow). The anomalous location of these genes may therefore reflect a need for their products during a phase of the infection intermediate between 'early' and 'late', or alternatively a requirement for separate control of their expression.

Finally, adenovirus genetics continues to thrive. In addition to existing *ts*, host-range and nonsense mutants, several groups have isolated deletion, duplication and insertion of mutants (T. Shenk, Connecticut; T. Grodzicker, Cold Spring Harbor; van Roy, Ghent). Many of these are located in parts of the viral genome which previously had been relatively refractory to genetic analysis as well as frustrating to the biochemists. Maybe these mutants will provide the inspiration needed to solve the conundrums set by the left-hand of the viral genome. □

Orthodoxy restored in neutrino physics?

from David J. Miller

Two neutrino conferences within two months: it seemed unnecessary when they were both announced but as it turned out sufficient had happened after the Purdue conference in May to give the Oxford conference during the first week of July a totally different direction. At Purdue the 'standard model' of particle physics seemed to be breaking down. In particular, a Gargamelle bubble chamber collaboration using the neutrino beam from the CERN SPS in Geneva had reported an unexpectedly high number of candidates for the weak neutral current process in which a muon-neutrino scatters from an atomic electron (see *News and Views* 273, 98; 1978; 273, 706; 1978). The number of these single-electron events was more than five times the number predicted by the standard Weinberg-Salam model of weak interactions, and the model was already under attack because of the non-observation of parity-violating interference effects in atomic physics.

Neither of these effects went away completely at the Oxford meeting but new data were presented from convincing and sensitive experiments which confirmed the standard model. At the same time evidence was reported to strengthen the experimental bases of the quark picture of hadrons and to indicate that quantum chromodynamics (QCD) may indeed be the fundamental

theory of the strong interaction.

The Gargamelle neutrino-electron scattering result was slightly revised from earlier published versions. Since the Purdue meeting the collaboration had scanned another 40% of their film and had developed more stringent criteria to test their candidate-events. At Oxford they claimed to see 9 single-electron events in approximately 35,000 normal muon-neutrino interactions of the charged-current type. The Salam-Weinberg model would predict only 2.5 to 3. But three other groups had new results which agreed with the model. A Columbia-Brookhaven collaboration using the Fermilab 15-foot bubble chamber had 108,000 charged-current events and saw only 11 single-electron candidates, very close to the predicted number. Two antineutrino experiments, each with approximately the same number of beam particles as the initial Gargamelle result, saw only 1 single-electron candidate between them where 3 were predicted. (BEBC bubble chamber; Bari, Birmingham, Brussels, Ecole Polytechnique, Rutherford Lab., Saclay, University College London collaboration. 15-foot bubble chamber; Berkeley, Fermilab, Hawaii, Michigan collaboration). If the Gargamelle result had been a true reflection of the theory then one might have expected even more candidates in the two antineutrino experiments than in Gargamelle.

Gargamelle has now been outvoted. There is a lot more film still to be scanned for these events, but it seems

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unlikely that the rate with either neutrino or antineutrino beams will change much from the Weinberg-Salam prediction. A lower energy neutrino-electron scattering experiment by Aachan and Padua at the CERN PS also agrees with the model.

While Gargamelle has been outvoted, the atomic physicists have been outflanked. One of the two non-neutrino talks to the conference was given by a member of the collaboration which has just done the 'experiment of the year' on the Stanford Linear accelerator in California (see *News and Views* 274, 11; 1978). With enormous care, and with multiple checks on the internal consistency of the data, they have studied the scattering of high energy electrons from a deuterium target and detected a difference of 1 part in 10,000 between the scattering rate for electrons with their spin pointing forwards and the rate for electrons with their spin backwards. This asymmetry is exactly what is predicted by the Weinberg-Salam model. P. Sandars (Oxford University) gave the other non-neutrino talk reviewing the atomic physics results. He reminded the conference of the absence of any parity violation in the observations of his own group and in the two separate experiments which have now been done in Seattle. He also mentioned a similar experiment in Novosibirsk. All four experiments have investigated the optical rotation of polarised laser beams by bismuth vapour. The Russians claim to see a clear parity-violating signal, but the full details of their techniques and their data have not yet been released. Sandars pointed out the great difficulties in calculating atomic matrix elements for such complex atoms as bismuth and suggested that clearer results may be obtained when experiments are done with hydrogen. Unfortunately these experiments are harder to do.

In a review of neutral current phenomena Sakurai wrote down a general formalism for all possible neutral-current processes and compared it with the whole range of data available at the conference; the new SLAC result, the single-electron rates, neutrino-proton and neutrino-neutron elastic scattering, single pion production, neutrino and antineutrino total cross sections and hadron energy distributions, as well as the old results on low-energy electron-neutrino scattering from electrons. The only parametrisation which satisfies the formalism and fits most of the data is identical to the prediction of the Salam-Weinberg model. The outstanding disagreement with this fit is the absence of parity violation in three of the four bismuth experiments. It is just possible that the

Stanford experiment and the null results on bismuth could both be correct, but many other experiments must then be wrong, as well as the Weinberg-Salam model.

In charged-current interactions there was a continued trend towards tidiness. Old disagreements between different experiments, such as the 'high y anomaly' which was only seen by the Harvard - Pennsylvania - Wisconsin-Fermilab group, have disappeared. Some very elegant analyses were reported, particularly that of data from old Gargamelle and new BEBC experiments in which the structure-functions for neutrino-nucleus scattering were extracted and shown to depart from the predictions of the naive quark model (CERN, Aachen, London (Imperial College), Oxford, Saclay collaboration). Instead of appearing to contain the same number and kind of quarks in all circumstances, giving rise to simple pointlike scattering at all angles, protons and neutrons scatter neutrinos in different ways when there is a big momentum-transfer to them than when there is a small transfer. This is analogous to the discovery that α -particle scattering did not obey Rutherford's formula for large momentum-transfers. QCD predicts the way in which the simple model should break down and the data presented at the conference indicate that the breakdown is of exactly the predicted kind. Similar breakdowns of the naive model in electron and muon scattering also fit the QCD predictions very well. It has even been possible to extract experimental values for some of the basic parameters of QCD from the neutrino data. This analysis, and many other separate experiments on charged currents, strange-particle production and charmed particle production, have confirmed the picture of the nucleon as an object made from three valence quarks, bound together by the QCD field of colour gluons. The gluons sometimes exist as virtual quark-antiquark pairs (called the 'quark-antiquark sea') from which neutrinos may scatter. Some kind of event, such as charmed particle production by antineutrinos, can only happen if one of the antiquarks in the sea has been hit. Thus it is possible to measure the number of sea quarks of the strange type by measuring the rate of charm production by antineutrinos.

The same BEBC collaboration that carried out the QCD analysis also produced the curiosity of the conference—a possible resonance between μ^- and π^+ at a mass of 1.8 GeV/ c^2 . They found it by chance when studying hadron resonance production in neutrino scattering on hydrogen. After enhancing the signal by a few selective cuts on the data they applied the same tech-

nique to their neon film and saw a similar structure. Each bump is of little more than 3 standard deviations in significance, so they are claiming nothing yet, but there was great speculation about what it might be. It certainly does not fit into any predicted scheme of particles.

The predicted particles which were discussed at length were those which might contain the new quark flavours—'top' and 'bottom'. 'Bottomonium' has been seen—the Υ (upsilon) and its excited states (see *News and Views* 273, 705; 1978). No one has seen any sign of the top quark, but many theorists seem convinced that it will be there. S. Glashow, in his final review talk, grouped the fundamental spin $\frac{1}{2}$ particles into three groups of four. There are the everyday particles, the up and down quarks, the electron and its neutrino; then a heavier set consisting of the strange and charmed quarks, the muon and its neutrino. After that it is sensible to expect another four, of which we have observed two, the bottom quark (in the Υ) and the τ lepton. So we expect a top quark and a τ -neutrino to come along soon. Seeing them may be difficult. It had been suspected that the new quarks or the τ lepton might be causing the three-muon events which have been seen by the big counter experiments (CERN, Dortmund, Heidelberg, Saclay; Harvard, Pennsylvania, Wisconsin, Fermilab; Caltech-Fermilab). But calculations have now been made which account for most of the trimuon events by adding low-mass muon pairs to ordinary charged-current events. They come either from pair production by virtual photons radiated from the final-state muon or by pair production by way of vector mesons in the hadron shower. There is little evidence for exotic new processes on top of these two relatively unexciting effects, both of which seemed to be visible in the data presented to the conference. A number of speakers, including Harari and Glashow, suggested that the new quark flavours could show up in neutrino interactions when beam energies are raised beyond the present limits of 200 to 300 GeV. This will have to wait for some years, at least until the 'energy-doubler' is built at Fermilab. The most likely signature for heavier quarks is an increase in the number of strange particles produced as, say, a top quark decays to a bottom quark which itself decays to a charmed quark and then a strange quark. The present generation of counter experiments could not observe such detail in the hadronic shower but in the New Techniques session some ambitious types of detector were suggested which might do so and at the same time provide more

target mass than a heavy-liquid bubble chamber like BEBC. □

Ninetyeast Ridge not aseismic after all

from Peter J. Smith

EVERY now and then one is brought up with a jolt as some long-accepted notion is destroyed at a stroke. Ninetyeast Ridge has just proved a case in point. This 4,500 km long ridge, which dominates the topography of the eastern Indian Ocean, lies roughly along longitude 90°E between about 32°S and 10°N, has a width of 50–100 km and rises an average 2 km above the surrounding ocean floor. The northern portion (to about 7°S) is broken up into a series of en echelon blocks whereas in the south the ridge is straight and flat-topped. But the most conspicuous characteristic of the ridge—conspicuous by its absence, that is—is its seismicity. Ninetyeast Ridge is commonly supposed to be a dormant fossil feature in the middle of the Indian-Australian plate. It is 'aseismic' in the sense that, unlike actively spreading ridges, it appears not to have associated with it frequent small earthquakes. Indeed, it is often cited as the archetypal aseismic ridge, the model for similar ridges elsewhere (for example, the Walvis Ridge in the south Atlantic).

But as Stein and Okal (*J. geophys. Res.* **83**, 2233; 1978) now point out at some length, Ninetyeast Ridge is not aseismic at all. On the contrary, historically it has been associated with substantial seismicity. A new compilation of seismic data by Stein and Okal shows that since 1913 the region has seen four earthquakes with surface wave magnitudes (M_s) greater than 7, ten with M_s in the range 6–7 and at least thirteen (and possibly as many as twenty-five) with body wave magnitudes (m_b) in the range 5–6. When large earthquakes are taken into account Ninetyeast Ridge, far from being aseismic, is very highly seismic (although the southern section is much less so). Indeed, it is far more seismic than any spreading ridge and has a seismicity comparable to that of large transform fault systems (for example, the San Andreas fault). In terms of large earthquakes, only subduction zones are more seismic than Ninetyeast Ridge.

To Stein and Okal this can only mean that Ninetyeast Ridge is either an active plate boundary or a zone of

major intraplate deformation. In fact they opt for the latter on the basis of a study of earthquake mechanisms and bathymetry. It will come as no surprise to most Earth scientists to hear that the tectonics of the Ninetyeast Ridge area are as complex as those in any other part of the Indian Ocean. But briefly, and perhaps over simply, the portion of the ridge north of 10°S is the main active seismic zone, where both vertical and strike slip motion occur. The latter is left lateral along a roughly north-south plane and is associated with compression across the ridge. In this northern section the ridge zone thus has some transform fault-like characteristics, correlating roughly with that part of the ridge broken into irregular blocks. It is not a true transform fault, however, because the compression occurs over a broad zone rather than precisely along the ridge.

South of 10°S, where the ridge is much smoother and much less seismic, the style of deformation differs on opposite sides of the ridge which itself remains largely undeformed. To the east normal faulting occurs, possibly related to the formation of a complex of ridges and graben-like troughs roughly parallel to the main ridge. These features suggest tensional deformation with tension in the NW-SE direction. To the west of the ridge, on the other hand, there seems to be NW-SE compression taking place largely aseismically. Here there is a complex terrain comprising NE-trending ridges and deeps. According to Stein and Okal this overall interpretation is generally consistent with the data and, if correct, implies that the Indian plate is currently undergoing substantial internal deformation with some relative motion between the Indian and Australian sides. In other words, the common assumption that the old Indian and Australian plates have been acting as a single plate for about the past 32 million years is not entirely valid.

Broadly it is as if the west (Indian) side of the plate is encountering resistance from the collision with Asia whereas the east (Australian) side is subducting smoothly at the Sumatra trench. The word 'broadly' is crucial here, however, for the detailed picture must be very complex indeed. Part of the difficulty in sorting it all out arises from the fact that the present geological pattern is not entirely the result of the present phase of tectonic activity but is partly a reflection of the ridge's much earlier history; current tectonic processes are only modifying a pre-existing structure. And this works both ways, of course. Stein and Okal claim that their results have no direct bearing on the question of the

origin of Ninetyeast Ridge which has been variously regarded as a horst, the result of overriding plates, a consequence of excess volcanism, and the trace of a mantle plume. But they also claim that "a significant fraction of the morphology of the Ninetyeast Ridge is due to the present-day tectonics of the area". Clearly, therefore, those interested in the origin of Ninetyeast Ridge must be careful not to include in their interpretations features that have developed much more recently as part of the ongoing deformation identified by Stein and Okal. □

Halogenated hydrocarbon effects

from Alastair Hay

IN the late 1960s, the polychlorinated biphenyls (PCBs) were recognised to be widespread industrial pollutants and to produce toxic symptoms in animals and man. They induce the skin condition chloracne, drug-metabolising enzymes, and porphyria and have immunosuppressive effects. Vos *et al.* (*Food and Cosmetic Toxicol.* **8**, 625 1970) were the first to report that commercial PCB formulations were contaminated with highly toxic polychlorinated dibenzofuran (PCDF) impurities. This contamination, they suggested, could complicate interpretation of toxicity studies of PCBs. Many observers now believe that it is these impurities which are responsible for differences reported in PCB toxicity studies. The PCBs, PCDFs and the polychlorinated dibenzodioxins (PCDDs) are all halogenated hydrocarbons and a meeting to review the toxic properties of this class of compounds was held recently in New York*.

Chemistry is central to any consideration of the biological properties of the halogenated hydrocarbons. Discussing the formation of the toxic polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), C. Rappe (University of Umea, Sweden) reported on the numerous processes which generate these compounds. It is well known that the PCDDs are produced when trichlorophenates and pentachlorophenates are heated. Another source has recently been identified; the fly ash and flue gases of incinerators in several European cities contain both the PCDDs and PCDFs.

Rappe suggests that pyrolysis of the polychlorinated diphenylethers will also produce furans and that this ther-

* 'The International Conference on Health Effects of Halogenated Hydrocarbons' organised by the New York Academy of Sciences was held in New York from 24–27 June, 1978.

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mal conversion is of the same order of magnitude as that from PCBs.

The problem of variability in analysis between different laboratories becomes particularly complicated when toxicity patterns are similar for two classes of chemicals, and this according to J. A. Moore (National Institute of Environmental Health Services, North Carolina) is the case for PCDFs and PCDDs. Looking at the effect of 2,3,7,8-tetrachloro, 2,3,7,8-tetrabromo and 2,3,4,7,8-pentachloro dibenzofuran in mice, guinea pigs and rhesus monkeys he noted species variations in the oral LD₅₀ values when the analogous halogenated dibenzodioxins were compared. Variations in the rates of metabolic degradation of halogenated aromatics were reported by H. B. Matthews (NIEHS) to depend on the degree and position of halogenation of the parent compound. Halogenated hydrocarbons are easily absorbed from the gut and lung. The more polar halogenated aromatics—phenols and phenoxyacetic acids—are excreted before metabolic degradation. The less polar PCDDs, however, are concentrated in the liver and excreted more slowly. Halogenated biphenyls—the least polar—accumulate in adipose tissue and skin and are generally metabolised before excretion. According to Matthews, as long as the 3,4 positions of the PCBs are available for enzyme attack the chemicals will have a fairly short half life.

R. A. Neal (Vanderbilt University School of Medicine, Nashville, Tennessee) observed that 2,3,7,8-tetrachlorodibenzo dioxin (TCDD) at a concentration of up to 1 mg per kg body weight did not prevent metamorphosis of tadpoles and neither did the dioxin interfere with the growth of human lymphocytes in culture. It has been reported that TCDD affects a hepatic cell line in culture, so Neal believes that it is just possible that the effect varies from cell to cell.

Both the PBBs induce hepatic and extrahepatic monooxygenases. Inducers of the hepatic enzymes have been classed in two groups with phenobarbital (Pb) and 3-methyl cholanthrene (3-MC) as the prototypes. According to J. Goldstein, (NIEHS) most PCB and PBB isomers belong to the Pb-type and induce cytochrome P-450; induction generally correlates with degree of halogenation and resistance to metabolism, but the position of the halogen is crucial in another respect. Isomers of these compounds with halogens in the meta and para positions but not in the ortho position (the PCDDs and PCDFs for example) are 3-MC types and induce a P450 subspecies, P448. This latter class is 25 times more toxic and potent than the Pb-type as in-

ducers. Toxicity symptoms also appear to correlate well with a chemical's potency as a 3-MC type inducer.

Poland *et al.* (*J. biol. Chem.* **251**, 4936; 1976) isolated a cytosolic binding protein for TCDD in mouse liver. Poland (University of Wisconsin) believes this to be a receptor for TCDD and consequently for other inducers. Toxicity symptoms also appear to correlate with a chemical's potency as a 3-MC type inducer. Poland (University of Wisconsin) believes this to be a receptor for TCDD and consequently for other inducers. Toxicity symptoms also appear to correlate with a chemical's potency as a 3-MC type inducer. Poland (University of Wisconsin) believes this to be a receptor for TCDD and consequently for other inducers. Toxicity symptoms also appear to correlate with a chemical's potency as a 3-MC type inducer.

Concern about the carcinogenicity of the PCDDs has been voiced many times at Séveso. There are two convincing studies which now report that the 2,3,7,8-tetrachloro isomer is a potential carcinogen. R. J. Kociba (Dow Chemicals, Michigan) in a study previously referred to (*see Nature* **269**, 749; 1977) reported, however, that it is a weak carcinogen when given orally. A. M. Shefner (Illinois Institute of Technology Research Institute) reporting similar findings to Dow says that both TCDD and a mixture of isomers of hexachlorodibenzodioxin are weak carcinogens if administered orally but that when applied dermally they are complete carcinogens.

The clinical symptoms for assessing the extent of exposure to halogenated hydrocarbons is still a problem. Now, however, there seems to be some agreement that the skin disease chloracne is one of the most sensitive indicators of toxicity. This is the view of J. S. Taylor (Cleveland Clinic, Ohio) and S. A. Fischbein (Mount Sinai School of Medicine, N.Y.), and J. J. T. W. A. Strik (Agricultural University, Netherlands) believes that as halogenated hydrocarbons will induce porphyria in experimental animals, examinations of the urinary porphyrin pattern is also an important screening test for workers exposed to these chemicals.

Hexachlorophene, probably the best known bactericide, has been used in maternity clinics for many years. This use has been questioned in a report by Halling (*Swedish Med. J.* **74**, 542; 1977), who claimed that the bactericide was responsible for an increase in the malformation rate of children born in Swedish hospitals where hexachlorophene was used. In a report accompanying this paper the Swedish Health Services said they could not substantiate the Halling hypothesis,

that a larger study was in progress to check, it, but that they did find the high incidence of malformations in the Gothenberg area—one of those studied—considerably higher than would be expected. They could offer no explanation for this, however.

Halling (Södertälje Hospital, Sweden), in a paper presented on her behalf, has now reported results for a larger retrospective study involving 10 hospitals, 460 women exposed to hexachlorophene and 233 controls. In the hexachlorophene group 25 severe malformations were observed in 460 neonates; in the 233 controls no severe malformations were seen. Minor malformations were also higher in the hexachlorophene group. The Halling study—which reports a wide range in the type of abnormalities seen—is, as yet, unconfirmed. One hopes that other investigators will act swiftly to confirm or refute it. □

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NATURE

A hundred years ago

THE MODE OF RECOGNITION AMONG ANTS.—The combats and communications of ants are among the most interesting and mysterious phenomena. The Rev. H. C. McCook has given an account to the Academy of Natural Sciences at Philadelphia of some experiments he has made to determine what is the mode of recognition among ants. He has studied the pavement ants (*Tetramorium caspium*), which he has observed engaged in continued combat for over a fortnight, the warriors being only the workers or neuters. There is no distinguishable difference between the ants of the fighting parties, yet they recognise each other infallibly as friend or foe. They challenge all comers with their antennæ; if they are friends, they pass on; if foes, they straightway interlock and "fall to." Mr. McCook surmised that recognition was based upon a certain odour emitted by the respective factions. He found that if they were enveloped in an odour of eau-de-Cologne, while not at all deprived of activity, all became harmonious; those who were previously engaged in battle unclasped one another, and they went on for several days amicably feeding, burrowing, and building.

From *Nature* **18**, 8 August, 386; 1878.

review article

Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukaemia

Leo Sachs*

A cell system has been developed to determine and dissect the controls that regulate normal myeloid cell growth, differentiation and malignancy, and to suggest a novel approach to the therapy of myeloid leukaemia.

STUDIES on the control mechanisms that regulate the growth and differentiation of normal cells can help in elucidating the origin and development of malignancy. Cells of the haematopoietic system, whose differentiation involves the induction of different cell lineages with various biochemical markers, seem to be particularly favourable material for such studies. Haematopoietic cells migrate normally to various parts of the body. The regulation of these cells may thus involve special control mechanisms, that may not necessarily be the same as those found with non-migrating cells which maintain intimate cell-cell contacts within a solid tissue.

To explore the nature of the controls that regulate the behaviour of normal cells and the breakdown of these controls that leads to malignancy, it is both advisable and convenient to study the growth and differentiation of specific cell types from single cells in culture under the influence of chemically defined inducers. Such experimental systems have been developed for the analysis of haematopoiesis¹⁻⁷. It has now been shown that all the main types of mammalian haematopoietic cell including macrophages, granulocytes^{1,4-9}, erythrocytes¹⁰, and B¹¹⁻¹³ and T lymphocytes^{12,14-16}, can be cloned in culture in the presence of appropriate inducers. I shall describe how cultured myeloid cells can be used to determine and dissect the controls that regulate growth and differentiation of macrophages and granulocytes and the development of myeloid leukaemia. The results also suggest a possible approach to therapy based on the phenotypic reversion of malignancy by the induction of normal differentiation in malignant cells. Unless otherwise stated, all the experiments described in this article have been carried out with cells from mice.

The myeloid cell regulator MGI

Normal myeloid precursors can be induced to grow and differentiate to mature macrophages and granulocytes by the protein inducer⁴⁻⁶ that we now call MGI (macrophage and granulocyte inducer)^{7,17}. MGI, which has also been referred to as mashran gm¹⁸, colony stimulating factor¹⁹, or colony stimulating activity²⁰, is specific for the induction of macrophages and granulocytes. The protein is secreted by and has been partially purified from various types of cell including fibroblasts and macrophages. The most complete purification has been from mouse fibroblasts giving a molecular weight of about

68,000 (refs 17, 21, 22), and from mouse lungs giving a molecular weight of about 23,000 (ref. 23). It is, therefore, possible that the lower molecular weight form may be a subunit of the higher molecular weight MGI, as may also conceivably be erythropoietin²⁴ (molecular weight 46,000; ref. 25), a protein which induces the growth and differentiation of erythrocytes. Purified MGI can induce the formation of macrophages and granulocytes. There may, however, turn out to be specific molecular forms of MGI that induce only macrophages or granulocytes, or there may be a requirement for different co-factors^{17,26} for differentiation to one or the other cell type. Incubation of the precursor cells with MGI for different periods of time has indicated that the presence of MGI seems to be required until the process of differentiation is completed^{5,27}. Normal myeloid precursors require MGI for cell viability, growth and differentiation²⁸. It remains to be determined whether these requirements are met by a single form of MGI.

Induction of normal differentiation of myeloid leukaemic cells by MGI

The discovery of MGI raised the question of whether this protein can also induce the normal differentiation of myeloid leukaemic cells. In order to test this possibility, leukaemic cells from different human and murine myeloid leukaemias were studied. The data indicate that there is one type of myeloid leukaemic cell in both humans^{9,29} and mice^{7,26,30} that can be induced to differentiate normally. Experiments with mice have shown that these leukaemic cells can be induced to differentiate not only *in vitro*, but also *in vivo*³¹. Differentiation *in vivo* can be enhanced by injecting MGI, or even better by injecting cells that produce MGI and can be regulated by cells involved in the immune response³¹. The experiments with mice have also shown that differentiation in these leukaemic cells to mature macrophages and granulocytes can be induced by purified MGI³² (Fig. 1) and that both mature macrophages and granulocytes can be derived from the progeny of a single leukaemic cell³³. This type of leukaemia from different strains of mouse has been cloned in culture³²⁻³⁵ and will be referred to as MGI⁺D⁺ (D⁺ for differentiation to mature cells)³³. Like normal macrophages and granulocytes, the mature cells induced from these leukaemic cells are no longer malignant *in vivo* and no longer multiply *in vitro*³⁶. Unlike mouse erythroleukaemic cells³⁷ that have lost the ability to respond³⁸ to the normal

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erythroid inducing protein erythropoietin, MGI⁺D⁺ myeloid leukaemic cells can still respond to the normal myeloid inducing protein MGI.

Isolation of the myeloid precursor cells from normal bone marrow³⁹, has made it possible to compare the sequence of differentiation in normal myeloid and MGI⁺D⁺ leukaemic cells. Both cell types were induced by MGI to produce the same mature cells, macrophages and granulocytes, in the same differentiation sequence. The sequence of differentiation in both cases was the induction of C3 and Fc rosettes⁴⁰, C3 and Fc immune phagocytosis³⁵, synthesis and secretion of lysozyme⁴¹ and the formation of mature macrophages and granulocytes^{9,33,35,36}. We have so far not been able to differentiate MGI⁺D⁺ cells into haematopoietic cells other than macrophages and granulocytes. It remains to be determined whether under other as yet undefined conditions, these leukaemic cells can also be induced to differentiate to other cell types, and in the meantime we cannot be sure that we are studying a malignancy originating in a pluripotent stem cell.

Origin and development of myeloid leukaemia

Although both the normal and MGI⁺D⁺ leukaemic cells could be induced to differentiate normally to macrophages and granulocytes by MGI, the normal cells differed from those that are leukaemic, in that the latter are viable and could grow in the absence of MGI whereas the normal cells required MGI for cell viability and growth²⁸. These leukaemic cells are, therefore, malignant not because they cannot be induced to differentiate by the normal inducing protein, but because they no longer require this protein for viability and growth. We therefore propose that an inadequate supply of MGI can occur *in vivo*, possibly because of a decrease in MGI synthesis or the production of inhibitors of MGI activity. This would then limit

the growth of normal cells without affecting the growth of leukaemic cells. A partial or complete loss of the requirement of this protein for viability and growth, may thus be the cause of myeloid leukaemia.

The MGI⁺D⁺ leukaemic cell studied did not have a normal diploid karyotype and the change in the requirement for MGI for cell viability and growth was associated with a chromosome abnormality^{42,43} both *in vitro* and *in vivo*. This suggests that the origin of leukaemia due to a loss in the requirement for MGI for viability and growth is genetic and due to a chromosomal change. It is still an open question whether the fundamental lesion in the myeloid leukaemias occurs in pluripotent haematopoietic stem cells, or in committed cells that may have more restricted development pathways. Chromosome studies have shown that MGI⁺D⁺ leukaemic cells without a completely normal complement of all the chromosomes can still be induced to develop a normal differentiation phenotype^{42,43}. The phenotypic reversion of malignant cells to cells with normal growth control but without a completely normal chromosome complement for all other genes, has also been found with other cell types^{7,44-46}. Studies with different types of myeloid leukaemic cells have indicated that the change which allows the leukaemic cells to grow in the absence of MGI can be followed by other genetic changes that produce blocks in the induction of differentiation by MGI.

Genetic dissection of the control of differentiation

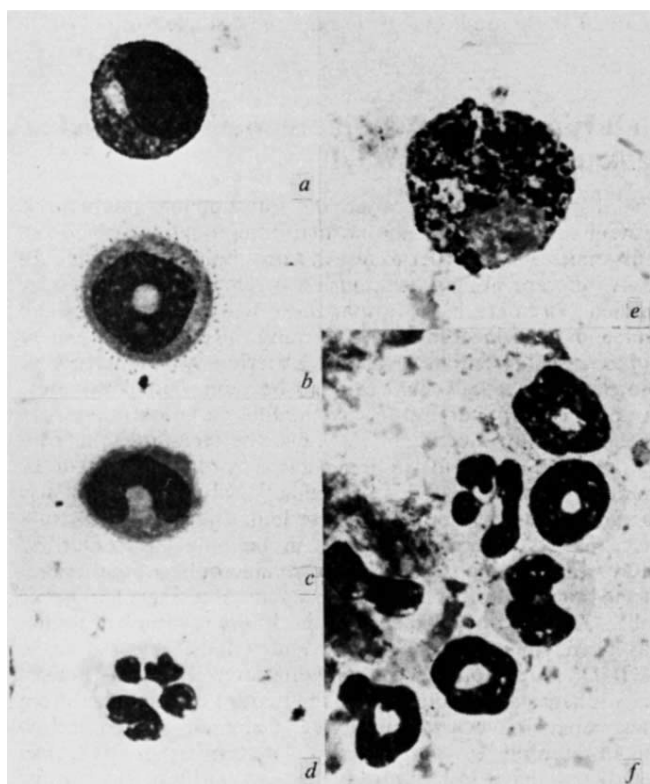
The isolation and study of mutants of myeloid leukaemic cells, all of which grow without added MGI^{33,35,40,41,47,48}, has shown that there can be blocks at different stages of differentiation and that there are separate controls for the induction of Fc rosettes, C3 rosettes, Fc immune phagocytosis, C3 immune phagocytosis, synthesis and secretion of lysozyme, formation of mature macrophages and the formation of mature granulocytes³⁵. Mutants have also been isolated which differ in their time of induction and sequence of differentiation. In one such mutant, MGI induced the synthesis and secretion of lysozyme without going through the stage of Fc and C3 immune phagocytosis³⁵. The isolation of other mutants should make it possible to define further not only the degree of independence of the control for each marker, but the extent to which other stages in the sequence can be omitted or sequences can be reversed and the cells still proceed to the next stage. It will also be interesting to determine if there are mutants that still require MGI for viability and growth but are blocked in differentiation, and whether such cells are malignant.

Other characteristics of normal cells that we have found to be induced in MGI⁺D⁺ cells by MGI include cell migration³², non-immune phagocytosis³⁶ and chemotaxis (G. Symonds and L. S., unpublished), synthesis of actin (B. Hoffman and L. S., unpublished), increase in β_2 -adrenergic receptors⁴⁹, agglutinability and stimulation of the pentose cycle by concanavalin A (Con A)⁵⁰, glycolytic production of ATP⁵⁰ and a reacquisition of the normal requirement for MGI for cell viability and growth²⁸. An analysis of protein synthesis by 2-dimensional gel electrophoresis has shown 73 protein changes after induction of MGI⁺D⁺ cells by MGI (B. Hoffman and L. S., in preparation). There are thus many other markers including a variety of enzymes found in normal mature cells⁵¹⁻⁵⁵ that can be used for the genetic dissection of the controls for normal differentiation in these cells.

Control of cell competence for the induction of normal differentiation

Our studies with myeloid leukaemic cell clones with different degrees of competence for the induction of normal differentiation by MGI have shown that competence was associated with membrane differences between MGI⁺D⁺ and the less

Fig. 1 Differentiation of MGI⁺D⁺ cells to mature macrophages and granulocytes by MGI. Undifferentiated blast cell (a), stages in the differentiation to mature granulocytes (b-d), macrophage (e) and group of granulocytes in different stages of differentiation (f).



competent cells. These include differences in Con A-induced cell-to-cell binding between a fixed and an unfixed cell^{56,57}, cell binding to nylon fibres coated with different densities of Con A⁵⁸, Con A agglutinability after ATP depletion⁵⁰, the frequency of free or specifically anchored Con A surface receptors as measured by capping⁵⁹ indicating differences in the cytoskeletal structures that can convey information from the membrane to the nucleus⁶⁰, cap formation by H-2 antigens (U. Bushkin and L. S., unpublished) and MULVg antigens⁶¹, differences in desensitisation of functional β_2 -adrenergic receptors possibly due to an alteration in the uncoupling system between adrenergic receptors and adenylate cyclase⁴⁹, and differences in the amount of surface ecto-ATPase activity⁶². We suggest that certain membrane properties may be associated with an appropriate arrangement of surface receptors for MGI or its internalisation, and that this may be required for induction of normal cell differentiation by this protein.

Cell competence for differentiation by MGI was also associated with differences in the production and inducibility of type C RNA virus, and the highest degree of competence was associated with the highest degree of virus production⁶¹. There may thus be an association of viral sequences with regulatory sites for differentiation. The increased virus production may indicate a different state of the regulatory sites for differentiation in the competent cells, and the higher virus production may either be a by-product of or directly influence these regulatory sites. This, therefore, seems to be a favourable system for investigating the role of type C virus in modifying cell competence for differentiation by a normal regulator. It will be interesting to determine the relationship between production of virus (or viral components) and the competence of cells to be induced to differentiate by MGI in type C virus containing myeloid leukaemic cells from man⁶³ and rat⁶⁴, and to compare the frequency of cells with various degrees of

competence for differentiation in myeloid leukaemias from different human and murine populations.

Isolation of segregants from the MGI⁺D⁺ to a less competent phenotype and vice versa has shown that the altered phenotype varies in stability. In many segregants the altered phenotype is unstable and after multiplication the cells soon revert to the parental phenotype, whereas in other segregants the new phenotype is stable for many cell generations. Chromosome studies have indicated that the stable, but not the unstable segregants, have specific chromosome changes that can be detected by chromosome banding⁴². A possible explanation is that competence for the induction of phenotypic changes associated with differentiation may be due to transposable genetic elements⁶⁵, the stabilisation of which requires the specific chromosome changes observed.

Karyotype analysis of cells with different degrees of competence for the induction of differentiation by MGI has indicated that the genes which control cell competence are located on mouse chromosomes 2 and 12, and that inducibility by MGI is controlled by the balance between these genes⁴³. We have suggested that these chromosomes also carry genes that control the malignancy of these cells⁴³. The malignancy of mouse myeloid leukaemic cells is suppressed when they are hybridised with normal macrophages⁶⁶, presumably because of a change in the balance of these genes⁷. Karyotype analysis in human myeloid cells has also shown specific chromosome changes in human myeloid leukaemia⁶⁷. It will be of interest to determine the behaviour of these leukaemic cells with different degrees of competence after inoculation into blastocysts, as has been done with teratocarcinoma cells⁶⁸⁻⁷⁰ and after culture on fat cells from the bone marrow⁷¹.

Induction of some normal cell markers by treatment with other compounds

Studies with various compounds, including some used in cancer therapy, have shown that certain stages of differentiation can be induced in appropriate clones of myeloid leukaemic cells by steroid hormones such as dexamethasone, prednisolone and estradiol^{40,47}, and by presumably surface acting agents such as the lectins Con A, phytohaemagglutinin, pokeweed mitogen (J. Lotem and L. S., in preparation), lipopolysaccharide, lipid A⁷² and dimethyl sulphoxide (DMSO)^{41,73}. Differentiation is also induced by actinomycin D and other compounds that can interact with DNA; for example, cytosine arabinoside, mitomycin C, 5-bromodeoxyuridine^{40,74} nitrosoguanidine and X-irradiation (A. Falk and L. S., in preparation), some polycyclic hydrocarbons (Z. Schwarzbard and L. S., in preparation) and the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (J. Lotem and L. S., in preparation). The induction of some stages of differentiation by low concentrations of actinomycin D^{40,74} may be explained by inhibition of the formation of repressor(s) of the differentiation process. Compounds that can induce some normal cell markers in an appropriate clone of MGI⁺D⁺ leukaemic cells are shown in Table 1. Not all the compounds induced the same markers or induced changes in the same clones. MGI was the only compound that induced all the changes to mature macrophages and granulocytes.

An example of the dissection of the controls for induction by a steroid inducer (SI) such as dexamethasone and MGI is shown in Fig. 2. It can be seen that MGI and SI do not induce the same markers even in clones (SI⁺MGI⁺) that respond to both compounds and that it is possible to isolate mutants that are SI⁺MGI⁻, SI⁻MGI⁻⁴⁸, or SI⁻MGI⁺ (L. Cohen and L. S., unpublished). The lack of response to dexamethasone in the SI⁻ clones was not due to any detectable defect in the number, nuclear transport, or association with DNA containing structures of the steroid receptors⁷⁵. The results indicate that there are different cellular sites for MGI and a steroid inducer such as dexamethasone. DMSO induced C3 but not Fc rosettes, and macrophages but not granulocytes, in a MGI⁺SI⁺DMSO⁺

Table 1 Inducers and non-inducers for normal cell markers in MGI⁺D⁺ myeloid leukaemic cells

Type of compound	Inducers	Non-inducers
Peptide hormones	MGI	Erythropoietin, nerve growth factor, insulin, ubiquitin, thymopoietin, interferon
Steroids	Dexamethasone, prednisolone, hydrocortisone, oestradiol	Progesterone, testosterone, epitestosterone, androstenedione, cortisone
Lectins	Concanavalin A, phytohaemagglutinin, pokeweed mitogen	
Polycyclic hydrocarbons	Benzo(a)pyrene, dimethylbenz(a)-anthracene	Benz(a)anthracene, dibenz(a, c)anthracene, dibenz(a, h)anthracene, phenanthrene
Other compounds	Lipopolysaccharide, lipid A, mitomycin C, dimethyl sulphoxide, cytosine arabinoside, hydroxyurea, thymidine, 5-iododeoxyuridine, 5-bromodeoxyuridine, 5-fluorodeoxyuridine, nitrosoguanidine, actinomycin D, adriamycin, daunomycin, X-irradiation, 12-O-tetradecanoylphorbol-13-acetate	Colchicine, vinblastine, Na butyrate, cycloheximide, dibutyl cyclic AMP, dibutyl cyclic GMP, cordycepin, deoxyglucose, ouabain, ionophore 23187

The different inducers were not all active on the same clone and did not all induce the same markers.

Cell type		SI ⁻ MGI ⁻ D ⁻		SI ⁺ MGI ⁻ D ⁻		SI ⁺ MGI ⁺ D ⁻		SI ⁻ MGI ⁺ D ⁺		SI ⁺ MGI ⁺ D ⁺	
Clone no.		1	8	6	7	5	11-M14	11-MS1	7-M9	11	12
Induction of:		SI  MGI	SI  MGI	 MGI	 MGI	SI  MGI	SI  MGI	SI  MGI	SI  MGI	SI  MGI	SI  MGI
Rosettes	C3		Spontaneous		+	+	+	+	+	+	+
	Fc		+	+	+	+	+	+	+	+	+
Immune phagocytosis	C3				+		+	+	+	+	+
	Fc				+		+	+	+	+	+
Lysozyme						+	+	+	+	+	+
Mature macrophages								+	+	+	+
Mature granulocytes								+	+	+	+

Fig. 2 Inducibility for differentiation-associated markers in different clones of myeloid leukaemia cells by the steroid inducer (SI) dexamethasone and the normal regulatory protein MGI. Clones that could be induced to differentiate to mature cells are referred to as D⁺.

clone. We have also isolated MGI⁺SI⁺DMSO⁻ clones^{41,73}, so that there seem to be different cellular sites for DMSO, SI and MGI. Partial differentiation of mouse erythroleukaemia cells can be induced by DMSO³⁷ and some other compounds⁷⁶⁻⁷⁹, apparently also via different cellular sites^{80,81}, although these cells have lost the ability to respond to erythropoietin. By contrast, the MGI⁺DMSO⁺ myeloid leukaemic cells can still respond to MGI.

Induction of MGI in MGI⁺D⁺ leukaemic cells

Among the compounds that induced differentiation to macrophages in some MGI⁺D⁺ clones were lipopolysaccharides (LPS) from different bacteria. LPS induced a high frequency of Fc and C3 rosettes, lysozyme and macrophages in certain clones of MGI⁺D⁺ cells, but not in MGI⁺D⁻ clones. Similar results were obtained with lipid A, so it seems that the active part of the LPS molecule is lipid A. Our results have also shown that treatment of the responding MGI⁺D⁺ clones with LPS or lipid A induced an activity in the conditioned medium that behaved like MGI⁷². This shows that appropriate clones of leukaemic cells can be induced to produce their own normal differentiation inducer. The induction of detectable MGI after treatment of MGI⁺D⁺ cells with LPS occurs before the induction of rosettes or lysozyme. These results suggest that the lipid A portion of LPS indirectly induces differentiation of MGI⁺D⁺ myeloid leukaemic cells by inducing in these cells production of the differentiation-inducing protein MGI⁷².

It will be of interest to determine which of the other compounds that can induce differentiation-associated properties in MGI⁺D⁺ leukaemic cells act directly and which like lipid A, may induce differentiation indirectly by inducing the production of MGI. The induction of regulatory proteins that can induce specific cell differentiation in the cells that differentiate, may be a more general mechanism for the induction of differentiation by various compounds in different cell types.

Possibilities for therapy

Our results suggest some novel possibilities for the therapy of myeloid leukaemia^{7,9,32,82}. The finding of MGI⁺D⁺ myeloid leukaemic cells that can be induced to differentiate normally by MGI suggests the use of MGI injection, stimulation of the production of MGI *in vivo*, or grafting of MGI producing cells, to induce the normal differentiation of these leukaemic cells.

This would be an alternative to cytotoxic agents, which kill normal cells as well as tumour cells. The membrane differences between cells which differ in their response to MGI may be useful markers to predict the response of the leukaemic cells to MGI *in vivo*. MGI⁺D⁺ leukaemic cells can be induced by MGI to require this protein again for cell viability and growth. This suggests that induction of differentiation of the leukaemic cells to this stage, followed by the withdrawal of MGI, may also result in the loss of viability and growth of the induced MGI⁺D⁺ leukaemic cells *in vivo*.

The induction of normal macrophage and granulocyte differentiation by MGI also suggests that injected MGI or MGI induced *in vivo* might be used to restore the normal macrophage and granulocyte population after cytotoxic therapy. MGI therapy may also be useful for treatment of non-malignant granulocyte diseases^{9,83,84}.

Our results can also help to explain the response of some, but not all patients, to chemical and irradiation cytotoxic therapy. We have shown that chemicals and irradiation used in therapy can induce some stages of differentiation in clones of myeloid leukaemic cells with the appropriate genotype, and that clonal differences in inducibility for normal differentiation-associated properties are not necessarily associated with differences in the response of these clones to the cytotoxic effect of these compounds. The induction in cells of Fc and C3 receptors, phagocytosis, and other macrophage-like properties, can be expected also to result in an altered cellular response to a variety of factors including antibodies. The growth *in vivo* of leukaemic cells with the appropriate genotype may thus be controlled by the therapeutic agents used not only for their cytotoxic effect, but because they induce these differentiation-associated properties. Differences in the competence of cells to be induced by these agents may thus explain differences in response to therapy in different individuals. The possible induction of MGI by these compounds may also play a part in the therapeutic effects obtained *in vivo*.

In summary, we suggest new forms of therapy of leukaemia based on the use of a normal regulatory protein such as MGI to induce differentiation in malignant cells and to induce a more rapid recovery of the normal cell population after the present forms of therapy. Alternatively it may be possible to use other compounds that can induce MGI *in vivo*, or can effect mutant malignant cells at differentiation sites that are no longer susceptible to the normal regulator. These possibilities may also be applicable to diseases in other types of cell, whose differentiation is controlled by other normal regulators.

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articles

Ice sheet topography by satellite altimetry

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The surface elevation of the southern Greenland ice sheet and surface features of the ice flow are obtained from the radar altimeter on the GEOS 3 satellite. The achieved accuracy in surface elevation is ~2 m. As changes in surface elevation are indicative of changes in ice volume, the mass balance of the present ice sheets could be determined by repetitive mapping of the surface elevation and the surface could be monitored to detect surging or significant changes in ice flow.

CHANGES in the volume of ice contained in the polar ice sheets on Greenland and Antarctica are intimately related to worldwide sea level and global climate. Because the sea level equivalent of the Greenland ice sheet is about 6 m and the Antarctic ice sheet about 60 m, ice volume changes of less than 1% are significant. The currently rising sea level (5 cm increase since 1940¹) might be caused in part by changes in the volume of the polar ice sheets. Although major changes in polar ice volume have occurred during the past 10,000 years, it is not known whether the present ice sheets are growing or shrinking, nor if their ice flow is stable or subject to surges². There is also

speculation that man's impact on the climate through fossil fuel combustion³ may induce ice-sheet melting.

Surveying the surface topography of the Antarctic ice sheet by satellite radio altimeter was proposed by Robin⁴ before the advent of radar altimeters designed for accurate measurements of sea surface topography. A direct determination of the mass balance of the ice sheets and monitoring them for surges by repetitive mapping with satellite radar altimetry at intervals of 5–10 yr was suggested by Zwally⁵. A change of only 1 m in the 2,000 m average elevation of the ice sheets would indicate a change in ice volume that is equivalent to 3 or 4 cm of sea level. It is not feasible to determine the mass balance of the major parts of the ice sheets to such significant accuracy by conventional surveying techniques or direct measurement of mass input (snow accumulation) against mass output (melting and iceberg discharge). The increased ice output in an ice sheet surge would certainly cause detectable surface elevation changes in the surge basin. Measurements of the surface topography and its time dependence could be used, along with ice dynamics models, to assess the existing potential for ice sheet surging and to determine the nature and extent of an actual surge if one occurs.

Extensive mapping of both the surface and bedrock topography of Greenland and Antarctica is being conducted by aircraft radio echo sounding^{6,7}. Recently, balloon-borne radar altimeters have also been used to map surface topography in Antarctica⁸. The accuracy achieved by these techniques, ± 30 m for aircraft and ± 60 m for balloons, is limited mainly by the uncertainty of the determination of the platform reference

level using pressure altimetry. At least one order of magnitude increase in the accuracy is needed to determine significant ice volume changes and details of the surface topography.

The geodynamics experimental ocean satellite (GEOS 3), currently in orbit, is the first of a series of oceanographic satellites equipped with a radar altimeter. The results presented here from analysis of GEOS 3 data collected over southern Greenland show that the radar altimeter determines the elevation of the ice sheet surface to an accuracy of about ± 2 m and delineates surface features of the ice flow. Detailed knowledge of the surface topography is important not only for assessing volumetric changes of the ice sheets, but as a primary input for study of glacier dynamics. In addition to the improved vertical accuracy provided by satellite altimetry, it can cover the ice sheets in a much shorter time.

Sensing system

GEOS 3 was launched into an orbit with a mean altitude of 844.5 km and an inclination of $114^\circ 52'$ on 9 April 1975. This orbit covers all water, ice and terrain between $65^\circ 08' N$ and S latitudes.

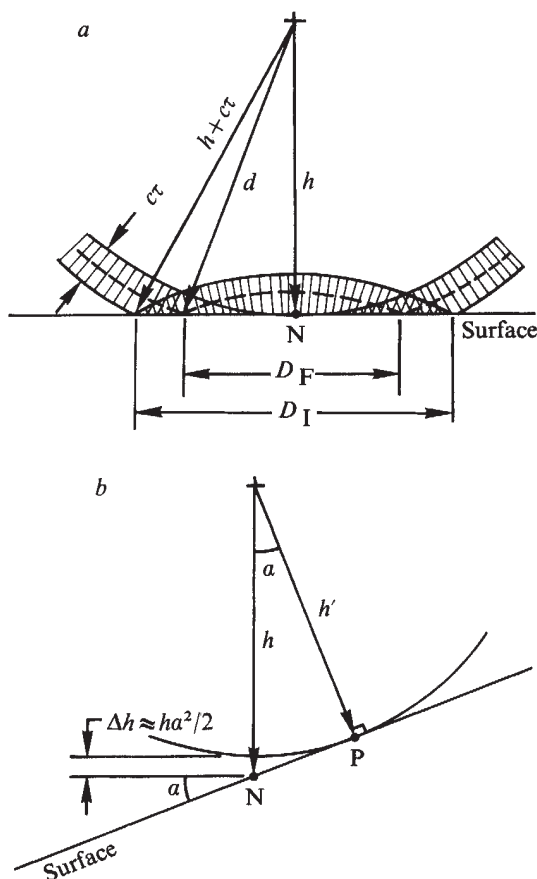
The altimeter height (h) is determined from the measured time between the transmission and return of 13.9 GHz radar pulses. The height measured is to the mean surface within the altimeter 'footprint', or effective backscattering area, which is determined primarily by the altimeter characteristics. Because the footprint size and location on the observed surface can affect the height measurement and the interpretation of the results, the footprint definition and the method of determining height from the return pulse must be carefully reviewed. The footprint size is limited by the width (τ) of the transmitted pulse. As the antenna is designed to illuminate an area larger than the footprint, the footprint is pulse-limited and not beam-limited.

The ideal pulse-limited footprint is defined as the maximum circular area, on a reflecting plane normal to a radial line from the altimeter, from which backscattering can be simultaneously received. It is derived as follows. The leading spherical wave front of a pulse transmitted at time $t=0$ first intersects the surface at time $t=h/c$, where c is the velocity of light. The circle of illumination then expands over the surface until $t+\tau$, at which time the trailing wave front intersects the surface as shown in Fig. 1a. After time $t+\tau$, point N at the centre is no longer illuminated. Backscattered signal at the altimeter is received from point N from time $2t=2h/c$ until $2t+\tau$. The distance d (see Fig. 1a), to the furthest point from which backscatter can also be received at time $2t+\tau$, is obtained from $2d/c=2t+\tau$. Thus, $d=h+c\tau/2$ and from geometry the diameter of the ideal footprint is $D_F=2(ch\tau)^{1/2}$, neglecting a second-order term. Effective pulse widths of 12.5 ns are achieved by pulse compression techniques in the short-pulse mode of GEOS 3 (ref. 9), and the corresponding footprint diameter is 3.6 km. The diameter D_I of the maximum circular area simultaneously illuminated, however, is a factor of $\sqrt{2}$ larger than the footprint.

The return signal increases from $2t$ to $2t+\tau$, and then remains nearly constant until the backscatter from the larger angles is attenuated by the beam limitations of the antenna, or is limited by the specular character of the surface. The altimeter split-gate tracker¹⁰ is designed to measure the time (T) from transmission to the centroid of the return signal rise, so that $h=c/2(T-\tau/2)$. The centroid is chosen primarily to minimise the effect of surface waves on the determination of the mean surface height, because surface waves tend to distort the signal rise symmetrically about the centroid. In fact, the significant wave height of ocean waves is derived from the slope of the return signal rise.

The height determined by the altimeter is not necessarily to the satellite nadir point (N), but is to the nearest surface within the field-of-view that reflects sufficient signal to the antenna. The GEOS 3 altimeter antenna has a 3db field-of-view of

Fig. 1 *a*, Radar pulse wave fronts intersecting a reflecting surface. The diameter, D_F , of the footprint (effective backscattering area) and the diameter, D_I , of the illuminated area are illustrated. *b*, Over a surface with slope α , the measured height, h' , is to the nearest reflecting point (P). The desired height, h , above satellite nadir (N) is about $h\alpha^2/2$ greater than the measured h' .



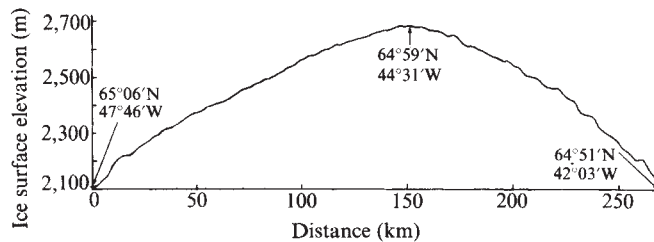


Fig. 2 Greenland ice sheet surface profile measured by the radar altimeter along orbit 0640 of GEOS 3. Vertical exaggeration is 134/1.

38 km (2.6° beam), which is about 10 times the footprint diameter. The antenna axis is also constrained to within 1° of nadir by the GEOS 3 stabilisation system. From a geometric standpoint, for a flat surface which has a mean slope (α) over the entire field-of-view, the nearest reflecting point (P) is upslope from nadir at a horizontal distance of $h \sin \alpha \cos \alpha \approx h \alpha$ and vertical distance of $h \sin^2 \alpha \approx h \alpha^2$ (see Fig. 1b) (ref. 4). However, the geometrical error Δh in the measured height (h'), compared to the desired height (h) above nadir, is a factor of two smaller and is equal to $h(1 - \cos \alpha) \approx h \alpha^2/2$. Also, the measured height is the true height at a horizontal distance $h(1 - \cos \alpha) \tan \alpha \approx h \alpha/2$ from nadir. In principle, for uniformly sloping surfaces the indicated surface can be adjusted by this

geometrical factor to obtain the true surface. For a surface slope of $1/300$ (see Fig. 3, for example), the true surface is located 1.4 km horizontally inward or 4.7 m vertically below the indicated surface. Where the slope does not extend across the entire field-of-view, the geometrical displacement is $< \Delta h$.

Ice surface elevations above sea level are computed by subtracting the altimeter height measurements from the calculated satellite altitudes above a reference ellipsoid of the Earth and also subtracting the geoid height (local sea level height above the ellipsoid). Geoid heights from the Marsh-Vincent model are used.¹¹ The precise position of the GEOS 3 satellite (latitude, longitude, and altitude above the reference ellipsoid) is determined from orbital solutions incorporating measurements from ground-based tracking systems. The accuracy of the resultant satellite position in three dimensions depends on the type of tracking data available, and it varies from ± 5 cm for laser tracking to a few metres for radar or doppler tracking.

Although an accuracy of a few metres is satisfactory for the horizontal position of the satellite, the altitude over the ice sheet is further refined by reference to the adjacent sea surface. For all the data shown here the elevation is adjusted to zero over the deep water east of Greenland where tidal and dynamic variations do not exceed 0.5 m. The data may be corrected later to compensate for the small tidal effects.

Height data points can be obtained from GEOS 3 at the rate of 100 s^{-1} or 10 s^{-1} , depending on whether the delays of individual pulses or the average of 10 pulses are analysed. Averaged data (10 s^{-1}) are used here so that each elevation data point represents an average along 0.67 km of the satellite ground track.

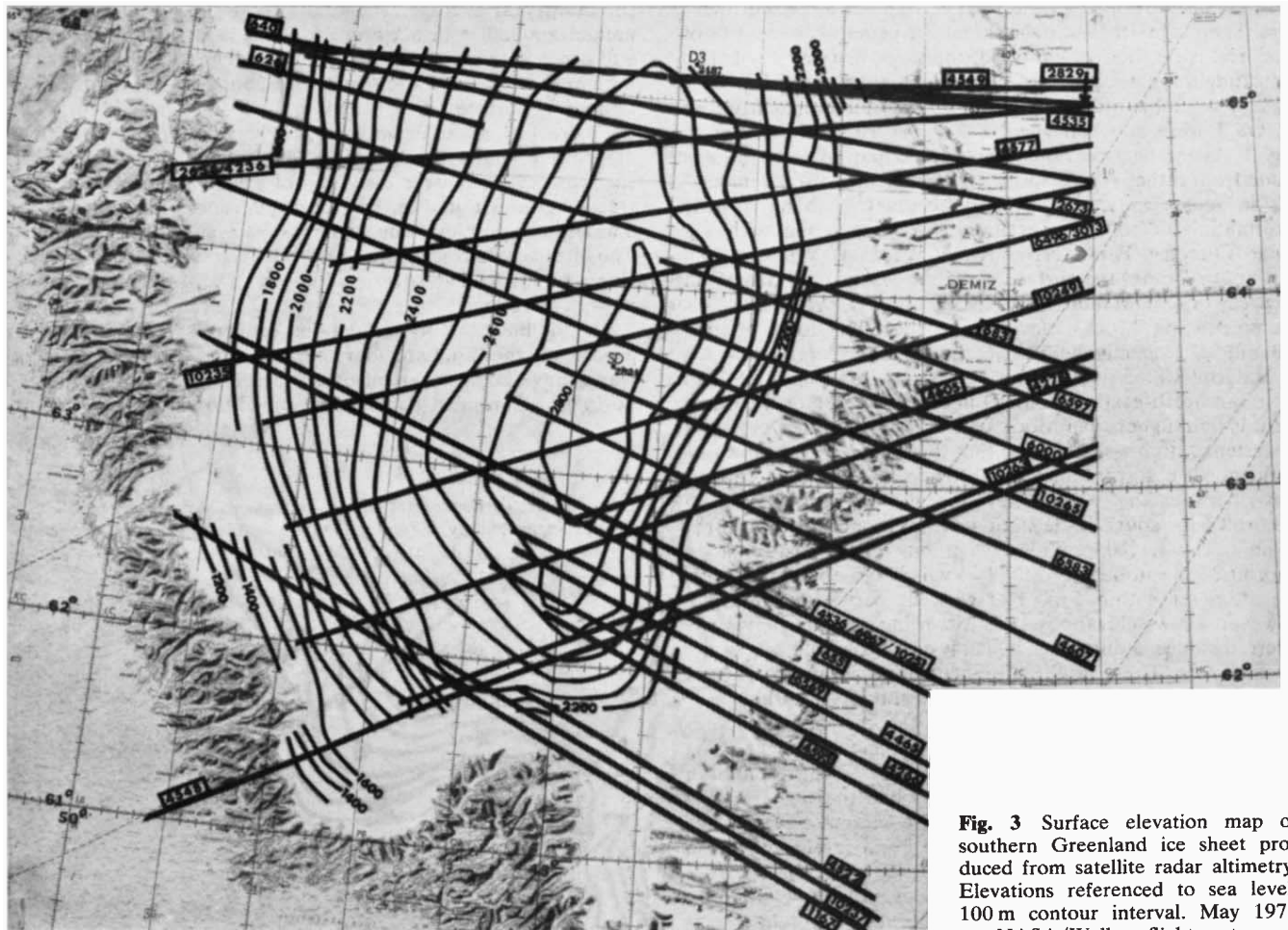


Fig. 3 Surface elevation map of southern Greenland ice sheet produced from satellite radar altimetry. Elevations referenced to sea level, 100 m contour interval. May 1978 NASA/Wallops flight centre.

Table 1 Observed elevations at intersections over ice sheet

Orbit no.	Date (yr month date)	Interval (d)	Position	Surface slope	Elevation above sea level (m)	Elevation difference (m)
640	750525		64.885°N,		2,310.22	
4236	760203	254	42.688°W	1/280	2,310.36	+0.14
683	750528		64.565°N,		2,283.75	
2658	751014	139	47.335°W	1/125	2,286.71	+2.96
683	750528		64.149°N,		2,636.45	
10235	770402	675	43.367°W	1/115	2,633.35	-3.10
2673	751015		64.814°N,		2,623.89	
4236	760203	111	43.898°W	1/370	2,621.97	-1.92
4548	760225		62.105°N,		2,559.95	
4593	760228	3	44.540°W	1/960	2,560.23	+0.28
10235	770402		63.468°N,		1,916.40	
10251	770403	1	48.557°W	1/110	1,916.33	-0.07

Analysis and discussion

The surface elevation segment in Fig. 2 from radar altimeter measurements taken on orbit 0640 of GEOS 3 shows a typical ice sheet profile. The surface along this segment is nearly symmetrical about the crest with a mean slope of 1/270 on each side. As the ice flow toward the edges of the ice sheet can be approximately described as plastic deformation, there is a well-known relationship between surface slope and ice thickness; in plastic flow, the shear stress and consequently the ice flow are proportioned to the surface slope and the ice thickness. Therefore, small surface slopes are generally indicative of thick ice. Near the 210 km point, the slope increases to 1/150 indicating thinner ice toward the mountainous east coast.

A topographic map prepared from altimeter data from 34 GEOS 3 orbits between May 1975 and April 1977 is shown in Fig. 3. As the best previous topographic map of this area was made from rather sparse surface altimeter measurements having in most cases only ± 30 m accuracy¹², there is little information of sufficient accuracy for comparison with the radar altimetry. However, at two locations (DYE-3 station, and South Dome) the surface elevation was established to an accuracy of several metres by Mock¹³ using navigational-satellite positioning. Mock's elevations at these locations, labelled D3 and SD respectively in Fig. 3, have been referenced to sea level. Orbit 10265 data show a maximum elevation of 2,833 m at 15 km north-east of South Dome. This altimeter measurement is 9 m higher than Mock's elevation of South Dome and consistent with his observation that the surface slopes upwards to the north-east from the South Dome site. Similar agreement exists between the DYE-3 elevation and the elevations from the orbit 6 km south of the station. In addition, Mock (personal communication) has provided elevations on the eastern slope of orbit 2658 south-east of DYE-3 which agree with the satellite altimeter within 5 m. The western part of orbit 2658, however, shows elevations 100–150 m higher than previously inferred values, a difference which is equal to about 5% of the ice thickness. These large differences occur in an area where the previous control points were sparse and less accurate.

The internal consistency or precision of the radar altimeter has been examined by analysing intersecting nadir tracks along the surface. Elevation differences at intersections presented in Table 1 range from 0.07 to 3.10 m. When the time interval between intersecting orbits is long, close agreement of surface elevations is not necessarily expected because accumulation, ablation, and ice flow can cause detectable variations. For the two intersections having time intervals of only a few days, the indicated differences are < 0.30 m. Because other intersects had intervals as long as 96 weeks, real elevation changes of the order of 1–2 m might account for part of those indicated

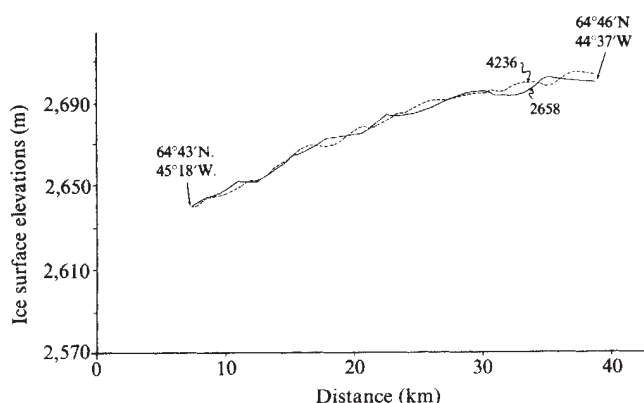
differences. The mean absolute difference is 1.4 m. Although additional analysis is required, these data are consistent with a precision of ~ 2 m or better.

A typical accumulation rate over southern Greenland is 0.55 m yr^{-1} ice-equivalent¹⁴ or about 1.5 m yr^{-1} of near-surface snow equivalent. If the ice flow is in equilibrium with the surface accumulation, then the vertical surface velocity is equal to the accumulation rate and the surface elevation will not change. Nevertheless, even if the ice flow is in equilibrium with the multi-year average accumulation rate, localised and inter-annual anomalies in accumulation rate and surface elevation will occur. For example, the effect of a localised doubling of a 0.55 m yr^{-1} accumulation rate for one year would be a temporary surface rise of 1.5 m.

Figure 4 shows an example of nearly overlapping tracks that are parallel and separated by 0.8 km across track. The mean surface elevations over distances of 10–20 km along the two tracks agree within 1 m, but local differences are as much as 5 m and are probably due to across-track surface undulations. The local across-track slopes are about the same as along-track slopes on the scale of several kilometres, indicating undulations in both directions.

The delineation of undulations or waves is an intriguing aspect of the altimeter-derived elevation profiles. Similar waves have been observed by surface levelling, for example, during the European glaciological expedition across Greenland

Fig. 4 Ice surface elevations on the eastern slope along parallel lines separated by 0.8 km. GEOS 3 orbits, 2658 14 October 1975; 4236 3 February 1976.



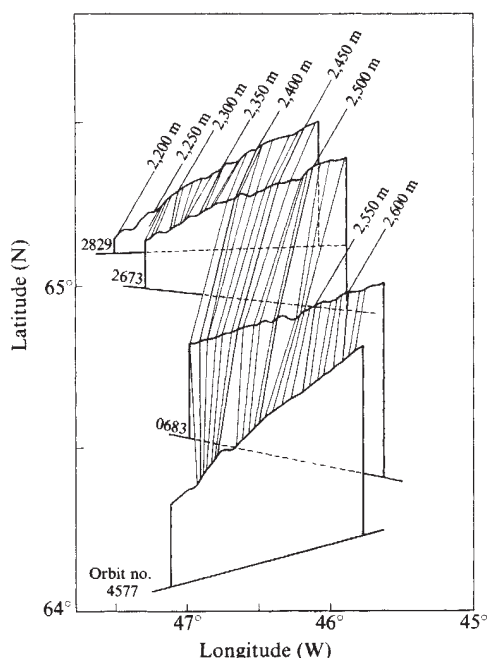


Fig. 5 Three dimensional view of ice surface on western slope of the ice sheet.

at about 70°N (ref. 15). The generation of surface undulations by ice flow over irregularities in the bedrock topography has been discussed by several authors including Budd¹⁶. Budd's theoretical analysis, and analyses of measured surface undulations with respect to bedrock undulations by Beitzel¹⁷ and Budd and Carter¹⁸, have shown that wavelengths about 2–6 times the ice thickness, that is wavelengths of the order of 5–10 km, dominate the surface profiles. Such waves generated by flow over the bedrock are stationary, whereas kinematic surface waves¹⁹ would move downslope and wind-drift accumulation waves²⁰ would move upslope. Therefore, bedrock-generated waves should be distinguishable from other surface waves by their stationary character.

Segments of four elevation profiles on the western slope are shown in Fig. 5. Waves in the western slope profiles shown here have individual amplitudes as large as 25 m. However, the eastern slope of track 640 in Fig. 2 shows more regular wave features. A series of about eight waves having an average wavelength of 10 km and an average amplitude of 15 m is apparent. The geometrical factors that limit the amplitude of the wave features in a radar profile have been described by Robin⁴. For example, for a wavelength of 10 km the curvature of the radar front is such that it will meet the crests of the surface waves at the same time that it meets the bottom of a valley 15 m deep at the mid-point. Thus, waves with amplitudes greater than 15 m having wavelengths of about 10 km apparently have 15 m amplitude. Therefore, the amplitude of the observed waves could be larger than indicated. On another scale, the average amplitude of wave features such as sastrugi, having small spatial extents compared to the altimeter footprint, should be derivable from the return pulse-shape as ocean significant wave heights are derived.

The detailed surface topography that will result from the analysis of the complete set of altimeter profiles will determine the three-dimensional character of the observed waves, and sequential mapping will determine their time-dependent nature. Because the wave character is related to the bedrock topography, ice thickness, and ice flow parameters, much can also be learned about ice sheet dynamics from the observation of surface waves.

Conclusion

Although the GEOS 3 radar altimeter was specifically designed for ocean measurements and the operating parameters are far from optimum for ice, it can be used for mapping the surface of the ice sheets. The accuracy of ~2 m obtained with GEOS 3 is sufficient to establish a baseline elevation map of the southern Greenland ice sheet for determination of its mass balance or state equilibrium between mass input and ice flow. This mapping will be repeated next by the radar altimeter on Seasat A launched in June 1978. The improved precision expected with Seasat A may reduce the time interval that is needed between mappings to detect significant elevation changes. Unfortunately, the Seasat-A orbit inclination of 108° only extends the coverage to ±72° latitude which is sufficient to cover ~50% of Greenland but only the coastal margin of the East Antarctic ice sheet. For example, it does not cover the very interesting West Antarctic ice sheet².

By using the data from closely spaced and overlapping orbits to remove the geometrical errors on sloping surfaces, it will be possible to establish more accurately the actual surface of the sloping portions of the ice sheet. Any residual errors in the absolute elevation due to slopes will have a smaller influence on the detection of elevation changes between repetitive mappings using the same technique.

The observation of numerous surface waves of the order of 10–20 m, amplitude and 10 km, wavelength corroborates surface measurements and will provide additional insights to the dynamics of large ice masses. This also points out the necessity for accurate mapping of the entire ice sheet, because extrapolation of localised surface measurements can be strongly influenced by localised or temporary features of the surface dynamics. For example, the determination of the locations of ice domes or ice divides requires the smaller scale undulations to be averaged to determine the mean surface.

The results from the analysis of intersecting tracks and the agreement with the surface control points implies that Fig. 3 is the most accurate topographic map of southern Greenland. One notable difference from the previous maps is the greater elongation of the southern dome as shown by the extension of the 2,600 m contour near to DYE-3 and the extension of the 2,800 m contour south to 62.7°N.

An altimeter similar to the present one but specifically designed for ice sheet mapping is required for future studies. The GEOS 3 studies are providing information on altimeter operation over ice and sloping surfaces that can be used to optimise antenna beam, pulse, sensitivity, tracking circuit, and other parameters and also to evaluate designs such as a multiple-beam altimeter. An appropriate ice altimeter placed in polar orbit at intervals of 2–5 yr would provide the data required to answer important geophysical questions about the growth or shrinkage and stability of the present day ice sheets.

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Composition and development of the continental tectosphere

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Beneath the old continental nuclei are thick root zones which translate coherently during plate motions. These zones are apparently stabilised against convective disruption by the depletion of the continental upper mantle in a basalt-like component. Construction of this delicately balanced tectosphere is accomplished by the dynamic and magmatic processes of the Wilson cycle.

THE model of lithospheric plate tectonics has provided a framework for rationalising a vast array of geological, geophysical and geochemical observations. Its essential elements are easily described in qualitative terms. Barrell and others in the early part of this century recognised that the mechanical behaviour of the outer layers to surficial loads can be portrayed adequately by a lithosphere overlying an asthenosphere. By its classical definition, retained throughout this article, the lithosphere is the region of the crust and upper mantle capable of statically supporting significant deviatoric stresses over geologically long periods, whereas the asthenosphere is the region below the lithosphere where the mass flows associated with isostatic adjustments occur. More recently, it has been shown that the Earth's surface is partitioned into a mosaic of plates which deform very little as they engage in large-scale horizontal motions. The region of the Earth occupied by these coherent plates is here termed the tectosphere. Plate motions are presumably the surface manifestations of gravitational instability induced by heat production within the interior. As new sea floor spreads laterally from its site of creation on the oceanic rift system, some of this heat is lost by conduction to the surface, and the temperatures within a thermal boundary layer decrease with time.

The essence of the lithospheric plate model is the assertion that the lithosphere, the tectosphere and the thermal boundary layer are identical in spatial extent, at least to the precision with which these terms are usefully defined. The need for the special term tectosphere, first used in this modern context by Elsasser¹ and Morgan² and later by Jordan³, is thus obviated. The model in its current stage of development is less specific about the nature of the sub-lithospheric mantle, but it is generally assumed that the asthenospheric material is well mixed by convective action, hence, chemically homogeneous, the dominant heat transport mechanism is advection, and the vertical temperature gradients are approximately adiabatic⁴. This lithospheric plate model has been quite successful in explaining oceanic tectonics, but can it be applied to the continents?

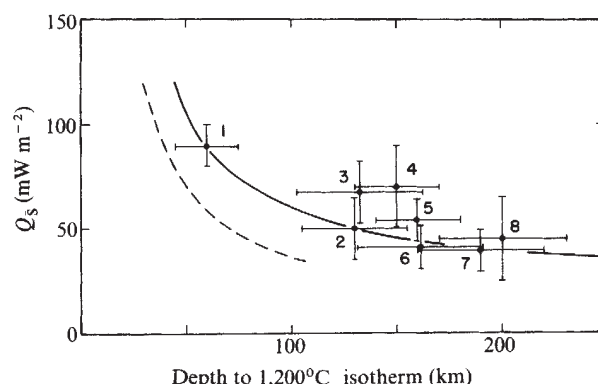
Continental thermal evolution

The first measurements of oceanic heat flow in the early 1950s suggested that the temperatures beneath the ocean basins exceed those beneath the ancient continental nuclei to considerable depth^{5,6}. Additional evidence supporting this view has since accumulated. Figure 1 shows the depth to the 1,200°C isotherm inferred from geothermometric data on mantle xenoliths plotted against the estimated surface heat fluxes for localities in Africa, Asia, Australia and North

America. The depth to the 1,200°C isotherm is about 200 km beneath the stable continental terrains (such as Yakutia, Kimberley) whereas the average oceanic temperatures at this level are significantly greater³. It is inferred from these and other data that, to 200 km depth and more, the vertical temperature gradients beneath the shields and stable platforms are significantly super-adiabatic and the horizontal temperature gradients in the transitions to oceanic structures are large^{3,8}. These inferences accord with the recent seismic data requiring substantial ocean-continent shear velocity and attenuation variations below a depth of 200 km^{3,9,20,21,51}.

Figure 1 also shows the well-known observation that the continental mantle is hottest in regions of recent magmatic activity (such as New South Wales, western USA) and apparently cools with time after the last major thermal event^{4,7,10,11}. Crough and Thompson¹¹ have used the thermal boundary layer hypothesis to formulate an approximate model describing this behaviour. According to their model, the continental temperature profiles decay by vertical conductive heat transport, as do their oceanic analogues, evolving to steady state with a time constant $\tau \sim Q^{-2}$, where Q is the heat flux from below the boundary layer. They advocate a value for Q in the range 23–33 mW m⁻², which yields 150 Myr < τ < 300 Myr. The locus of the Crough–Thompson model for $Q = 33$ mW m⁻² is shown in Fig. 1. This high value of the background heat flux predicts geotherms which are too steep and are difficult to reconcile with the available geothermometric data. To satisfy these data requires $Q < 15$ mW m⁻², implying a

Fig. 1 Continental surface heat flow plotted against depth to 1,200°C isotherm. Data points are regionally averaged heat fluxes^{42,43} versus 1,200°C depths for palaeo-geotherms constrained by xenolith petrology. These palaeo-geotherms are assumed to be representative of present-day thermal conditions. Localities, age of xenolith emplacement and references are: 1, New South Wales, Australia (Quaternary)⁴⁹; 2, Montana, USA (late middle Eocene)⁴⁴; 3, Eastern Mojave, USA (post-Miocene) (A. L. Boettcher, unpublished); 4, Navajo Volcanics, USA (late Oligocene)⁴⁷; 5, Northern Lesotho (late Cretaceous)^{35,46}; 6, Frank Smith, S. Africa (late Cretaceous)³⁹; 7, Kimberley, S. Africa (late Cretaceous)⁴⁰; 8, Yakutia, USSR (late Palaeozoic)⁴⁸. Error bars are subjective estimates. Solid line is locus of Pollack and Chapman's⁸ conductive steady-state models. Dashed line is locus of Crough and Thompson's¹¹ thermal boundary layer model for $Q = 33$ mW m⁻² (their equation (4)).



larger decay constant ($\tau > 700$ Myr) and a thicker steady-state boundary layer. The Pollack-Chapman⁸ geotherms form a locus consistent with these constraints, and they are therefore used in subsequent calculations.

Assuming that the thermal boundary layer hypothesis was correct; then, the mass excess from thermal contraction integrated over the boundary layer would increase smoothly with time along a curve similar to Fig. 2. This mass excess is large, and, since it is not evident in the global free-air anomaly field¹², it must be isostatically compensated for all times, presumably at shallow depths as in the oceans. However, the behaviour implied for the crust is inconsistent with the known history of the continents. For example, cooling from a geotherm with a surface heat flow of 150 mW m^{-2} to one with 40 mW m^{-2} generates a mass excess $> 9 \times 10^6 \text{ kg m}^{-2}$ below 40 km depth (Fig. 2). The compensation of this mass by the crustal column requires a crustal thickening of nearly 20 km. Although some thickening associated with thermal contraction has occurred locally in continental interiors (such as cratonic basins), in orogenic zones and along the margins^{11,13}, this model is vitiated by the existence of the exposed shields.

Furthermore, the base of the thermal boundary layer extends well below what can properly be called lithosphere. Studies of the topographic response to temporal changes in surface loads^{14,15} and the correlation between existing surface loads and the gravity field^{16,17} indicate that the effective thickness of the continental lithosphere for time intervals > 10 Myr is < 100 km. Hence, significant horizontal and vertical temperature gradients must exist within the continental asthenosphere. If the mantle is chemically homogeneous, these thermal gradients are accompanied by density gradients, and the sub-continental asthenosphere cannot be in hydrostatic equilibrium. For the configuration to be in static equilibrium requires deviatoric stresses to be maintained with magnitudes of the order of the vertical load differences^{18,19}. These load differences exceed 10^7 Pa (100 bar) for the thermal models considered here (Fig. 2) and cannot be maintained without invoking a rheology for the mantle inconsistent with the surface loading data. Alternately, mass flow must occur, but such flow will generally tend to establish hydrostatic equilibrium, quickly destroying any large super-adiabatic thermal gradients below the continental lithosphere.

A stabilising mechanism

From these considerations, it seems that the lithospheric plate model in its simplest form does not account for the existence or evolution of a thick super-adiabatic (conductive) layer beneath the old continental nuclei. To establish a more satisfactory model, we retain the assumption that the tectosphere and the conductive layer are identical but abandon the identification of tectosphere with lithosphere³. From the seismic data^{9,20} and the heat flow data⁸, the thickness of the continental tectosphere must be highly variable; it is thin (< 100 km) in unstable regions such as the western USA but probably reaches depths of the order of 400 km beneath the stable shields and platforms.

The previous discussion has emphasised the need for a mechanism to stabilise the large sub-lithospheric thermal gradients within the continental tectosphere against convective disruption. I have postulated the existence of chemical variations^{3,21}. Compositional gradients are envisaged to be adjusted by dynamic processes so that the tectosphere extending below the lithosphere is in approximate hydrostatic equilibrium with its surrounding mantle; the *in situ* densities at a given level beneath continent and ocean are equal and any thermally produced mass excesses within the sub-lithospheric continental tectosphere are locally cancelled by chemically produced mass deficiencies. By this hypothesis, the mineralogical assemblages within the thick continental root zones are less dense than those beneath the oceans if referenced to the same pressure and temperature.

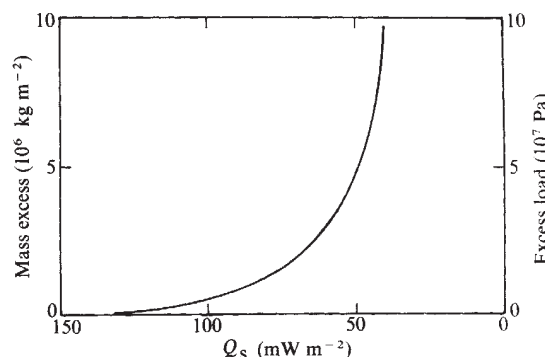


Fig. 2 Mass excess and corresponding vertical load difference due to thermal contraction in sub-continental mantle as functions of surface heat flow. Curve derived from Pollack and Chapman's⁸ geotherms assuming geotherms decay from an adiabat with potential temperature of $1,300^\circ\text{C}$ and slope of $0.5^\circ\text{C km}^{-1}$. Mass difference $-\alpha\rho\Delta T$ is integrated between 40 km depth and intersection of geotherm with adiabat using $\rho = 3,400 \text{ kg m}^{-3}$, $\alpha = 3 \times 10^{-5}^\circ\text{C}^{-1}$. ($10^7 \text{ Pa} = 100 \text{ bar}$).

Basalt depletion hypothesis

A simple and attractive mechanism for generating these density differences is available: the chemical gradients stabilising the thermal gradients can be induced by the removal of a basaltic magma from the mantle^{22,25}. Mantle mineralogy in the depth range 70–400 km approximates that of a garnet lherzolite^{26,27}, and the subtraction of basalt from garnet lherzolite leaves a residue less dense than the parental material^{23–25}. If the degree of basaltic depletion has a negative correlation with temperature, the continental tectosphere can be in hydrostatic equilibrium with its surrounding mantle. The requisite relative density decrease is $-\alpha\Delta T$, where α is the coefficient of thermal expansion and ΔT is the ocean–continent temperature contrast. In the depth range 150–200 km, the average temperature contrast between the ocean basins and shields probably lies in between 300 and 500°C (refs 3, 8). Thus for $\alpha = 3 \times 10^{-5}$ per $^\circ\text{C}$ the hypothesis requires a compositionally induced density difference between -0.9 and -1.5% at these depths.

To test this hypothesis, a simple algorithm for estimating the mineral compositions and densities of garnet lherzolites from their whole rock oxide composition has been devised²⁵. The mineral composition of four phases (garnet, clinopyroxene, orthopyroxene, olivine) are calculated from the seven major oxide components (SiO_2 , Al_2O_3 , Cr_2O_3 , FeO , MgO , CaO , Na_2O ; total Fe computed as FeO) by specifying various apparent distribution and partition coefficients. The densities of the mineral phases and the whole rock are computed assuming the additivity of end-member molar volumes. The whole rock density obtained by this normative scheme, denoted $\hat{\rho}$, is the density estimate for a garnet lherzolite assemblage equilibrated at mantle conditions ($\sim 5 \times 10^9 \text{ Pa}$, $1,200^\circ\text{C}$) but measured at standard conditions (10^5 Pa , 25°C). The present application requires only the density differences of various compositions at fixed pressure and temperature; hence, the corrections for elevated pressures and temperatures are second-order in small quantities and can be ignored.

The normative density algorithm has been applied to the available whole rock analyses of garnet lherzolite xenoliths from kimberlite pipes and to various model compositions of the oceanic upper mantle²⁵. The mean oxide composition of 78 xenoliths is listed with its normative parameters in Table 1; this average continental garnet lherzolite (ACGL) is similar to the estimates of other authors^{28–30}, although more samples with a greater geographical distribution are used here. Most of the xenoliths are from South African localities, but pipes in the USA and USSR are also represented. The ACGL is an approximation to the average mantle composition beneath the

Table 1 Parameters of an average continental garnet lherzolite (ACGL) compared with model compositions of the oceanic upper mantle

Composition (wt %)	ACGL*	Pyrolite ³¹	St Paul's Rocks (wt avg) ³²
SiO ₂	45.55 ± 1.45	45.30	44.63
TiO ₂	0.11 ± 0.06	0.71	0.29
Al ₂ O ₃	1.43 ± 0.60	3.55	3.78
Cr ₂ O ₃	0.34 ± 0.14	0.43	0.52
FeO	7.61 ± 0.94	8.48	8.15
MnO	0.11 ± 0.02	0.14	0.12
MgO	43.55 ± 1.98	37.60	39.40
CaO	1.05 ± 0.77	3.09	2.67
Na ₂ O	0.14 ± 0.10	0.57	0.34
K ₂ O	0.11 ± 0.10	0.14	0.10
Norm (wt %)			
Gt	6.0	12.3	15.3
Cpx	4.5	16.3	11.3
Opx	22.5	13.5	11.9
Ol	67.0	57.9	61.5
Normative density (kg m ⁻³)	3,353	3,397	3,401

* Average of 78 analyses of kimberlite xenoliths²⁵; oxide composition expressed as sample mean ± sample standard deviation.

shields and stable platforms in the depth range 150–200 km (ref. 25). Two model compositions for the oceanic mantle are compared with the ACGL in Table 1, Ringwood's pyrolite³¹ and a weighted composition of St Paul's Rocks³². Relative to these compositions, the ACGL is depleted in normative garnet and clinopyroxene, and its whole rock Fe/Fe+Mg ratio is lower, suggesting basaltic depletion of the subcontinental upper mantle (Table 1, Fig. 3). The depleted major-element chemistry of the mantle sampled by the xenoliths has been noted by many petrologists, most explicitly by Ringwood^{31,26} and Nixon and Boyd^{33–35}.

Because it is depleted in basaltic components, the ACGL is less dense than the model oceanic garnet lherzolites by about 1.3% ± 0.2%. This density difference is sufficient to offset the volume contraction resulting from a cooling of about 400 °C, which falls in the range of the ocean-shield temperature contrasts estimated from thermal models. Thus, one implication of the basalt depletion hypothesis is substantiated.

Another implication provides a more stringent test. Beneath the continents, in regions of large super-adiabatic gradients, the degree of basaltic depletion should decrease with depth coherently with the decrease in the ocean-continent temperature contrast. This is, in fact, observed³⁵, as shown in Fig. 4. The normative densities for various xenoliths in Fig. 4 correlate negatively with the Ca/Ca+Mg ratios in the clinopyroxenes. Since these ratios decrease with temperature^{37,38} and temperature increases with depth, the data in Fig. 4 indicate a rise in normative density with depth caused by increases in Fe/Fe+Mg and normative garnet. The magnitude of this rise agrees well with the theoretical curves based on the basalt depletion hypothesis (Fig. 4).

The model of a thick basalt-depleted continental tectosphere is also consistent with the seismic observations of substantial ocean-shield shear velocity and attenuation contrasts in the upper mantle^{3,9,20,21,51}. Temperature is probably the dominant factor in controlling the lateral variations in shear velocity, but chemical depletion may also contribute to establishing high mantle shear velocities in the cratons. The mean atomic weight is lowered by basalt removal, which increases the shear velocities^{50,25}, but, more importantly, basalt depletion raises the mantle solidus; for temperatures near the solidus, this can substantially elevate the velocities and reduce attenuation⁵⁷.

Isotope and trace element problem

The hypothesis of a basalt-depleted continental tectosphere agrees well with several independent lines of geophysical and petrological evidence, but it apparently conflicts with trace element and isotopic data. Continental basalts, for example, consistently display ⁸⁷Sr/⁸⁶Sr ratios which are higher and ¹⁴³Nd/¹⁴⁴Nd ratios which are lower than those of mid-ocean ridge basalts^{52,53}, and geochemists have inferred that the subcontinental mantle is considerably more enriched in large-ion lithophile (LIL) elements than its oceanic counterpart. Brooks, James and Hart⁵² extend this reasoning to argue for the existence of a continental tectosphere (or, in their terminology, lithosphere) "that is far from depleted, and which, under favourable thermal conditions, would be a ready source of basaltic magma." Clearly, from the previous discussion, this conclusion is favoured by neither the geophysical nor the petrological data.

The model advocated by Brooks and others is one interpretation of the available isotopic data, but it is by no means unique; there are tenable explanations which preserve the basic hypotheses stated here. For example, the ultimate source regions for the continental flood basalts, which provide much of the isotopic data, probably lie below the tectosphere in a more fertile layer of the mantle. This (hot) fertile material may intrude the tectosphere during episodes of continental rifting, when most flood basalts are produced.

Nevertheless, we must admit the possibility that the continental tectosphere is actually depleted in basaltic components (low Al₂O₃, FeO, CaO relative to MgO) but is enriched, at least locally, in LIL minor elements (for example, Rb, Sr, Cs, Ba, U, Th). This configuration is suggested by data from the basalt-depleted garnet lherzolite xenoliths themselves; those analysed show high ⁸⁷Sr/⁸⁶Sr ratios and low K/Rb, K/Sr, K/Cs and K/Ba ratios^{54,58–61}. LIL enrichment of previously depleted mantle could result from metasomatic processes involving the flux of volatiles or highly differentiated liquids^{54–56}. In fact, given the great age and complex evolutionary history of most tectospheric columns, extensive metasomatism is quite plausible; once depleted in basaltic components, the tectosphere is less likely to be the site of further large-scale melting, and it provides a ready trap and long-term reservoir for the very mobile mantle constituents.

Aspects of continental evolution

Previous investigators have attributed the substantial contrasts between continental and oceanic tectonics to the differences in crustal structure⁶². These differences are certainly important and probably explain why continental lithosphere is generally more easily deformed than oceanic lithosphere and why continental seismicity is more diffuse. But crustal structure is only part of the story.

It is proposed here that irreversible basaltic depletion of the upper mantle is crucial in regulating continental development and cratonic stability; that is, a fundamental coherence between surface tectonic behaviour and upper mantle compositional structure is postulated. Once depleted, low-density residual peridotite cannot be easily remixed with undepleted mantle. A depleted tectosphere is stabilised at lower temperatures, its solidus is elevated and its effective viscosity is thereby greater than undepleted mantle. These properties should drive the horizontal shear straining associated with plate motions to greater depths and isolate the crust from the thermal perturbations and mass motions associated with small scale convective disturbances. The existence of a 'decoupling zone' of concentrated horizontal shear strain beneath the continental tectosphere remains problematic, as is the distribution of driving forces, but the coherent motions of thick continental roots are more plausible if convection involves the lower mantle. This concept is in accordance with other data^{21,63–65}. Clearly, in the context of this model, continental drift involves more than just the passive rafting of sialic crust on an otherwise uniform lithosphere. If the model is

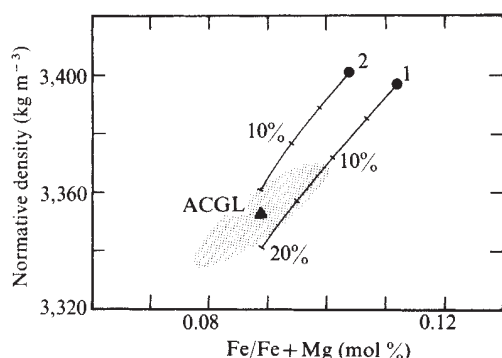


Fig. 3 Normative density compared with whole rock Fe/Fe + Mg ratio for average continental garnet lherzolite (ACGL) and two oceanic upper mantle model composition: 1, pyrolite³¹; and 2, weighted average of St Paul's Rocks³². Shaded field contains 67% of the samples used to derive the ACGL. Curves show displacements of oceanic model compositions by subtraction of indicated molar percentages of basalt²⁵; pyrolite-olivine basalt (ref. 26, Table 4-2) and St Paul's Rocks-average oceanic tholeiite³⁶.

credible, a full understanding of mantle dynamics will require the inclusion of forces due to compositional gradients into the governing equations. (Such forces are often encountered in physical oceanography; indeed, there is a notable analogy between the salt of the oceans and the basalt of the Earth.)

A thick, cool, depleted tectosphere capped by a mature continental crust is envisaged to be the eventual consequence of the magmatic and dynamic processes intrinsic to the Wilson cycle of rifting, drifting, closure and collision. This basic plate tectonic cycle has evidently operated throughout the Phanerozoic and the Proterozoic^{66,67}, and probably well back into the Archean^{68, 69}. For a tentative sketch of tectospheric evolution, there is no need to deviate from actualistic statements of the fundamental processes. Obvious temporal differences, such as the contrasts between Archean and modern tectonics, seem to arise from progressive tectospheric growth and associated crustal stabilisation⁶⁸.

In the current continental environment, basaltic depletion of the uppermost mantle occurs in two primary settings: during the magmatic episodes associated with continental rifting, especially by the extrusion of massive continental flood basalts⁷⁴, and in the mantle wedge above subduction zones⁷⁰⁻⁷³. (Averaged over Phanerozoic time, the latter site seems to have produced a much greater volume of basaltic rock.) Once significant depletion has occurred, the mantle is resistant to subduction and can support lower temperatures without convective recycling. Volatile flux or highly differentiated magmas from either subducted slabs or the deep mantle can impregnate the tectosphere with mobile LIL constituents and modify the details of its trace-element chemistry without inducing large-scale melting.

But geometrical⁷² and petrological^{26,73} constraints indicate that magmatic events efficiently deplete the mantle in basaltic constituents only to depths of 100–200 km. Some residual peridotite could diapirically rise from the descending slabs to augment the depleted wedges above subduction zones⁷⁵, but the protocontinental tectosphere produced in island-arc environments or along the active margins is probably thin, chemically heterogeneous and poorly consolidated. In the eventual course of the Wilson cycle, however, the dispersed regions of depleted mantle and their superjacent crustal columns are swept up and accreted to the primary continental masses.

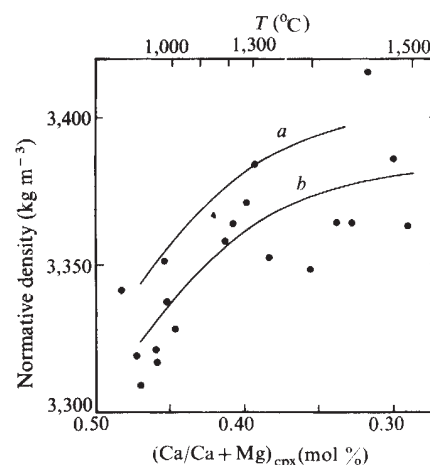
Further consolidation and thickening of the tectosphere result from the major compressive events at convergent boundaries, particularly episodes of continent-continent collision. The history of the crust following a continental collision has been analysed by Dewey and Burke⁷⁶. During very violent collisions of Atlantic-type and Andean-type margins, such as the one occurring today between India and Asia, the crust of

the Andean-type margin is compressively thickened by a factor of two or more, leading to the uplift of a Tibetan-like plateau. Dewey and Burke envisage this process of compressive crustal thickening to involve deformation essentially continuously distributed over a broad zone. If this occurs, it should be concomitant with a proportional thickening of the subcrustal tectosphere by the lateral and downwards advection of depleted peridotite. In this way, the thick root zones of the stable shields and platforms could be formed.

Thus, depletion, consolidation and thickening are the important constructive processes in continental tectospheric development. However, their iterative application over many Wilson cycles may be necessary to stabilise a thick tectosphere in any one region, because these constructive processes must compete against destructive processes. Whereas large-scale basaltic depletion is nearly irreversible, consolidation and thickening of the tectosphere are not. Any significant heating of a cool, thick, cratonic tectosphere by conduction from its periphery, by diapiric intrusion or by internal heat sources results in its thinning and dispersal by the upwards and lateral flow of low-density depleted material. Such motions may have an important role in episodes of continental rifting and breakup. Extension within the Basin and Range Province and the uplift of the Colorado Plateau may exemplify the consequences of an instability induced by tectospheric heating.

It is hypothesised that, throughout geological history, continental growth and stabilisation have been regulated by tectospheric development; that is, tectospheric depletion, consolidation and thickening are the fundamental chelogenic processes of the Wilson cycle. Presumably, magmatic differentiation before and during the Katarchean (>3,000 Myr BP) was very active, leading to the localised formation of primitive sialic crust^{69,78} and generating a large volume of depleted mantle. However, the existing continental tectosphere was probably thin, perhaps because mantle convection was more vigorously driven by an increased rate of radiogenic heat production⁷⁷. The diachronous stabilisation of the nuclear Archean cratons in

Fig. 4 Normative density plotted against Ca/(Ca + Mg) in clinopyroxene. Temperatures correspond to the diopside-enstatite solvus of Nehru and Wyllie³⁸. Points represent garnet lherzolite xenoliths for which both whole rock and clinopyroxene analyses are available²⁵. Theoretical curves are based on the hypothesis that basaltic depletion exactly cancels the density increase due to temperatures lower than the oceanic adiabat. To construct curves *a* and *b*, temperatures from Pollack and Chapman's⁸ 40 mW m⁻² geotherm were subtracted from oceanic adiabats with potential temperatures of 1,300 °C and 1,500 °C, respectively, and slopes of 0.5 °C km⁻¹. Normative densities were constructed assuming a pyrolite composition for the oceanic upper mantle ($\rho = 3,397$ kg m⁻³) and a thermal expansion coefficient of 3×10^{-5} °C⁻¹; these densities are plotted against temperatures on the continental geotherm using the upper scale. The increase in normative density with equilibration temperature observed for the xenoliths supports the basaltic depletion hypothesis.



the interval 2,800–2,000 Myr BP (ref. 78) is inferred to mark the earliest formation of thick tectospheric root zones with long-term stability. This development may be responsible for the fundamental transition from Katarchean permobility to Proterozoic linear (geosynclinal and mobile belt) tectonics.

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Palynology, palaeomagnetism and radiometric dating of Flandrian marine and freshwater sediments of Loch Lomond

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Loch Lomond in Scotland was part of the sea not only in late Devensian times but also in the middle Flandrian. Deep water cores from the southern basin show sediment with marine plankton and low remanent magnetism (RMN) between freshwater sediments. Raised beaches, deltas and estuarine flats were formed during the Flandrian transgression which ^{14}C dating shows to have lasted some 1,450 yr from 6,900 to 5,450 yr BP. Palaeomagnetic matching enables an intercomparison of Windermere and Lomond ^{14}C timescales and implies that both may be in error.

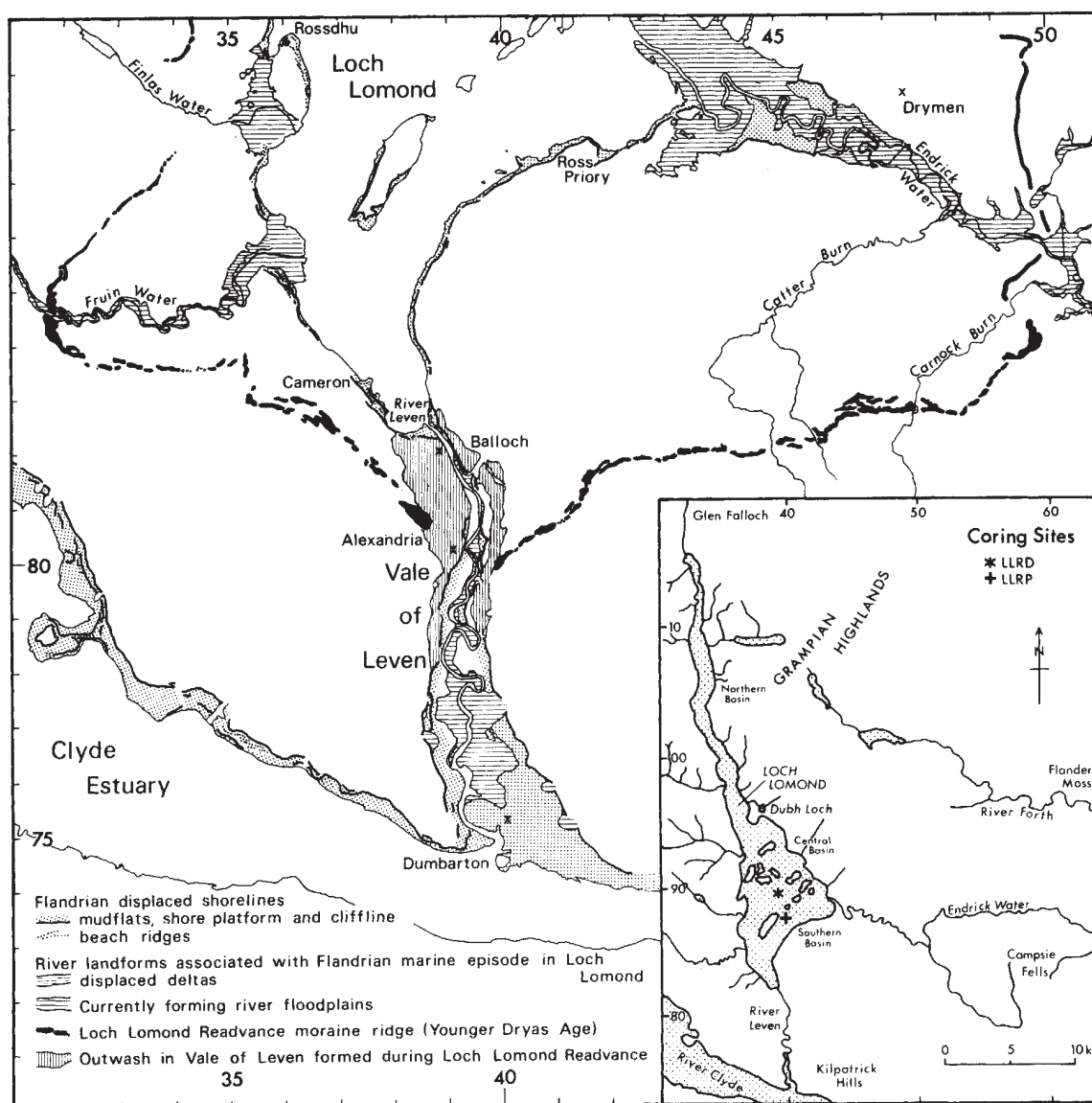
LOCH LOMOND takes the form of a north-south trending glacial trough¹ which is deep and narrow at the north where bedrock is predominantly mica schist and schistose grit, and shallow and wide in the south where the bedrock is Old Red Sandstone and Carboniferous sandstones, marls and basalts. In the highland zone the main rivers such as the Falloch, Douglas and Fruin drain into the northern and western sides of the loch, whereas in the lowland zone the main river is the Endrick Water which drains into the south-east corner of the loch from a large catchment that includes much of the Kilpatrick Hills and the Campsie Fells (Fig. 1, inset). The River Leven forms the outlet to Loch Lomond and drains southwards through the Vale of Leven to the Clyde estuary at Dumbarton. The water level in Loch Lomond is about 8 m o.d.

Loch Lomond, a warm monomictic lake, is divided into three basins: northern, central and southern. Oligotrophy is least extreme in the southern basin but even there exposure reduces the development of macrophytes, and reedswamp is notably absent. The shores of the loch have an intermittent fringe of *Alnus glutinosa* sometimes with *Myrica gale*, which is also apparent on the islands. Relict *Quercus* woodland, much modified after centuries of management², occurs along the eastern shore and much less so in the west where there are stands of *Betula* woodland. To the north in Glen Falloch, the loch almost reaches the area of former dominance of *Pinus sylvestris*, where there is a relict stand and stumps in shallow peat. Apart from long-planted conifers on the islands, there are, on the eastern slopes, recent large plantations of conifers, above which blanket peats are extensive, as on the western slopes. The hedgerows and fields on the richer ground at the southern end of the loch contrast markedly with the rough grazing, infested with *Pteridium*, on the rugged ground to the north.

Late Devensian glacial and shoreline history

Radiocarbon dates and related Pleistocene stratigraphy indicate that the southern part of Loch Lomond was ice-free by 12,500 yr BP (ref. 3), and that ice wastage was accompanied by a marine incursion which reached a maximum elevation of about 33 m o.d. During this submergence a large body of fossiliferous marine clayey silt (Clyde Beds⁴) was deposited. Sea level then fell to about 12 m o.d., at which level the prominent main late glacial shoreline⁵ was formed in the southern part of Loch Lomond. In turn, this event was closely followed by the Loch Lomond re-advance which occurred between 11,700 and 10,500 yr BP according to ¹⁴C dates from late glacial marine shells in Loch Lomond⁶ and organic detritus in the glacier catchment zone on Rannoch Moor⁷. The Loch Lomond glacier extended as far south as Alexandria in the Vale of Leven⁸, where a moraine ridge and outwash fan were constructed, and a barrier was formed between Loch Lomond and the Clyde estuary. In terms of subsequent marine activity

Fig. 1 Flandrian displaced shorelines in Loch Lomond and the adjacent part of the Clyde estuary. Note that this figure does not show late glacial shorelines or sediments. Scale and orientation of the main map are indicated by National Grid coordinates. The position of displaced shorelines on the Loch Lomond islands and certain parts of the Vale of Leven, where earthworks have since destroyed landforms and obscured sediments, are reproduced with permission of the Institute of Geological Sciences.



in Loch Lomond this barrier is important because it controlled the level of the freshwater loch, and the level that the sea must reach before it could re-enter the Loch Lomond basin.

Flandrian shoreline landforms

Flandrian shoreline landforms consist of shore platforms and cliffines cut into glacial deposits, late glacial marine clayey silt, and Old Red Sandstone sandstones and marls; and beach ridges formed of gravel (Fig. 1). Beach gravels also cover much of the shore platform and exhibit characteristic shore-process properties such as a high degree of roundness, a well sorted particle size distribution, and a predominant dip of the particles towards the water body. The shoreline levels vary around Loch Lomond according to shore configuration, fetch and glacio-isostatic deformation, but in the southern part fossil shorelines are developed at 13, 12 and 9 m o.d. Glaciofluvial landforms at several areas such as Rosdhu, Cameron, Balloch and Ross Priory (Fig. 1) extend down to the highest shoreline landform, above which they are unmodified by shoreline processes. This indicates that the highest observed shoreline landforms also form the marine limit. The Flandrian age of these features is determined by their stratigraphic relationship to glacial deposits of Loch Lomond re-advance age; and a marine origin, as opposed to a lake origin, is confirmed by their continuity through the Vale of Leven and into the Clyde estuary (Fig. 1). The shoreline at about 12 m o.d. in the southern part of Loch Lomond is exceptionally well developed and a rock-cut platform and cliffine may be as wide or as high as 160 m and 15 m respectively. This exceptional size is attributed to the exhumation, along particular stretches of coastline, of the already well developed main late glacial shoreline.

Raised deltas and estuarine flats are associated with the raised shorelines at the mouths of some of the rivers which enter Loch Lomond (Fig. 1). At the mouth of the Endrick Water, for instance, a well developed terrace is to be found above the present floodplain. The morphological properties of the terrace indicate that it formed as a delta with an intertidal extension, while the sea level in the area remained at 12 m o.d. A raised delta is also developed in relation to the same shoreline at the mouth of the Fruin Water, but at some of the steeper gradient rivers, such as the Finlas, there is no evidence for this fossil river landform, and it is assumed that any fossil features have subsequently been buried by the aggrading rivers.

Coring and cores

Using the University of Edinburgh Geophysics Department 6-m Mackereth sampler a series of cores was obtained from the southern basin at ~NS 385 900 under 24 m of water (LLRD1-3) and at ~NS 393 875 under 26 m of water (LLRP4) (Fig. 1, inset).

The cores are all longer than 4.70 m and exhibit similar stratigraphy shown in broad outline for core LLRD1 (Fig. 2). At the base of the core from 4.94 to 4.70 m there is grey clay. Brown silty clay from 4.70 to 3.75 m is interrupted by a thin layer of clay (3.90–3.92 m). The darker silty clay from 3.75 to 3.05 m contained a valve of the mollusc *Macoma calcarea* (Gmelin). The fine lamination, seen nowhere else in the core, from 3.05 to 2.78 m contained a small twig of *Alnus*. From 2.78 to the top of the core there is brown silty clay. Pollen analysis of a 1 m frozen finger core has helped to confirm that the Mackereth cores are nearly or entirely complete.

The pollen record

Pollen analysis of core LLRD1 (Fig. 2) was made difficult by sparse and poorly preserved pollen and may not be pursued below 4.20 m where the pollen is very infrequent.

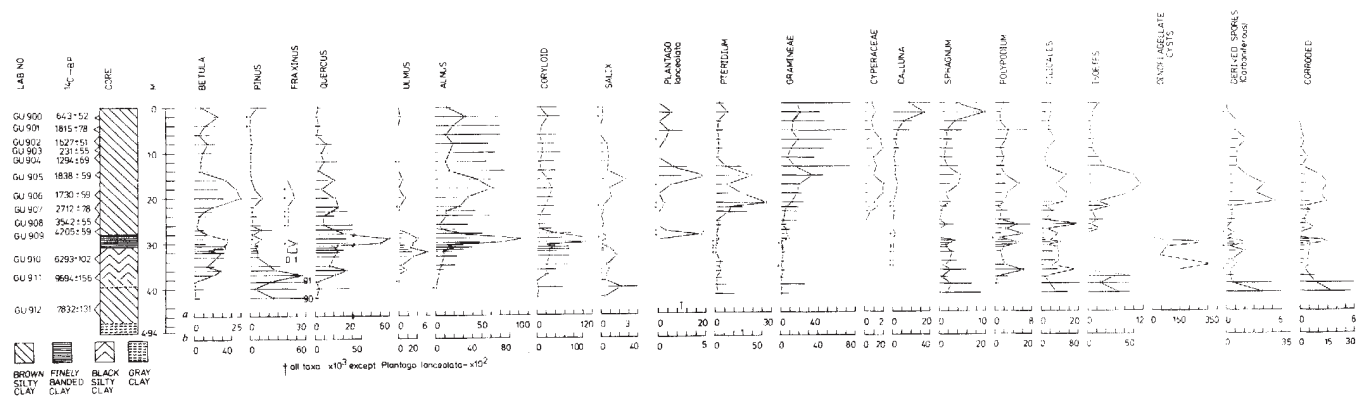
4.94–3.60 m: Low altitude sites in central and southern Scotland are characterised by very low values of pine pollen even in early Flandrian layers. In contrast are the very high values of pine in the Loch Lomond core (Figs 2 and 3). As in sites in upland Perthshire and Stirlingshire⁹, the Dubh Loch diagrams show pine peaks more or less coincident with the *Alnus* rise but even these are low compared to the Loch Lomond values. An explanation may be differential concentration and preservation of large amounts of pine pollen brought down by the River Falloch from expanding forests in the surrounding catchments.

3.60–3.15 m: The *Alnus* rise is taken to be between 3.60 and 3.50 m; at the former depth *Alnus* is less than 10% AP while at the latter more than 20% (Fig. 2). *Quercus*, *Alnus*, and *Betula* are the predominant tree pollen types. Herb pollen and spores are insignificant, apart from Filicales and *Polypodium* as in the lower levels.

3.15–0.20 m: The increase in pollen concentration in the finely laminated band of the core (Fig. 2) is very striking, especially with respect to *Alnus* but all the trees and tall shrubs show increases, some of them large. Good preservation and the total absence of the normally abundant corroded grains contrast markedly with the rest of the core. The *Ulmus* decline is taken to be between 3.15 (10% AP) and 3.00 m (1% AP). As at Flanders Moss, 20 km to the east¹⁰ and at Dubh Loch, the *Ulmus* decline is unaccompanied by any pronounced occurrence of agricultural pollen; *Plantago lanceolata* was first found at 2.90 m. There is a marked and sustained rise of grass pollen at 2.60 m (to about 20% AP) and again at 1.60 m (to about 40%, with values up to 75%). There is a very high value of Filicales spores at 2.70 m (75%), and there are high values of *Pteridium* spores between 2.40 and 2.20 m and again at 1.60 and 1.40 m.

Below 1.60 m, tree pollen concentration only once goes below 50% (coinciding with the highest *Pteridium*), whereas

Fig. 2 Pollen diagram from top 4.20 m of core LLRD1. Pollen values are expressed as *a*, concentration per cubic centimetre (vertical bars) by addition of exotic standard; and *b*, percentages (horizontal lines) of arboreal pollen (AP). Counts are mostly based on 150 AP. + = <1% AP.



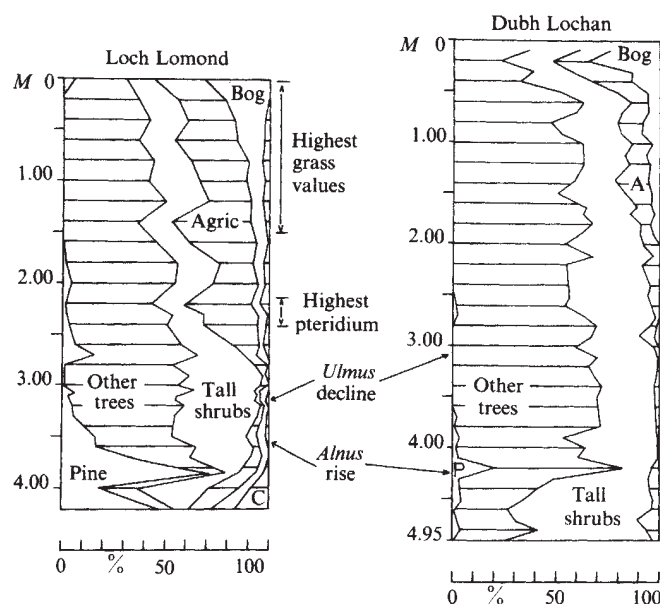


Fig. 3 Summary pollen diagrams from core LLRD1 and Dubh Loch Mackereth core. Pollen sum for LLRD1 is trees, tall shrubs, agriculture indicator plants (*Graminae*, *Plantago lanceolata*, *Pteridium*), bog plants (*Calluna*, *Cyperaceae*, *Sphagnum*) and derived Carboniferous spores. Pollen sum for Dubh Loch is identical but there were no derived spores. The Dubh Loch analysis comes from a 4.95 m long core, from under 11 m of water (the deepest point) of the small loch, the surface of which is at ~20 m o.d.

above that level it is always below 50% (Fig. 3). Clearly these high NAP pollen spectra result from human alteration of the vegetation in the catchment. Judging from the grass percentages, the most substantial changes, beginning about 1,700–1,800 yr BP, compare with the extensive clearances at Flanders Moss in the Roman Iron Age and at Bloak Moss, Ayrshire¹⁰, possibly somewhat later in the early Dark Ages.

Above 1.60 m and especially above 0.80 m *Calluna*, *Cyperaceae* and *Sphagnum* occur at their highest values, which may be explained by the inwash from eroding blanket peat.

Above 0.20 m, the topmost sample has 5% AP of *Pinus* pollen, following nine samples with exceedingly low concentrations, <1% AP. This rise of pine pollen is even more impressive in the frozen finger core, and apparently results from pine afforestation in recent centuries.

Derived spores: the core is unusual, if not unique, among British Flandrian deposits in containing throughout its length thick-walled, heavily ornamented, trilete spores, typical of Carboniferous rocks. The genera *Vallatisporites*, *Lycospora* and *Punctatisporites* have been recognised. These spores may have been carried into the Loch by the River Endrick, from areas of Carboniferous bedrock.

Palaeomagnetism

Using the methods described by Thompson¹¹ palaeomagnetic relative declination, inclination, intensity and susceptibility were determined (Fig. 4). Palaeomagnetic ages were assigned to the Loch Lomond sediments by (1) correlation of declination oscillations with carbon dated oscillations in Lake Windermere, (2) correlation of declination and inclination with palaeomagnetic records from kilns, tiles and pottery, and (3) correlation with magnetic data from observations (Table 1).

The Loch Lomond palaeomagnetic record shows a finer structure than can be seen in records from other British lakes, and clearly shows known characteristics such as the sharp turning point (*f*) and the broad, almost double turning point (*e*) which exist in records from Lake Windermere¹² and Lough Neagh¹³. The intensity and susceptibility changes follow one another closely and can be reproduced in remarkable detail

from core to core; in all cores the marine sediments have very low intensity and susceptibility.

In the post-marine sediment more than 14 characteristic fluctuations in intensity and susceptibility are found and can be used for accurate, between core, correlation. As found in Lough Neagh¹⁴, there is broad correspondence between susceptibility and grass pollen values. A similar explanation, involving soil instability and increased erosion and input of primary particulate substrate incorporating titanomagnetite, can be proposed for the source of the susceptibility increases. The high correlation of the susceptibility and intensity logs support a detrital or post detrital origin for the stable remanence. Detailed investigations (to be reported later) of anhysteretic and isothermal remanences following the method of Levi and Banerjee¹⁵ do not lead to a simple estimation of relative geomagnetic palaeointensity.

Radiometric dating

Radiocarbon analyses were performed using the CO₂ gas proportional method^{16–18}. Low carbon contents necessitated relatively long depth increments of half cores to be used for assay of the organic fractions of 13 samples. Radiocarbon ages (Figs 2 and 5) were calculated using the conventional Lamont VIII formulae¹⁹ and the 5,568 yr Libby half-life value. Stable isotope corrections were applied using mass-spectrometric analyses of each sample. The errors quoted are based solely on the counting uncertainties in radioactivity measurement.

The plot of ¹⁴C age against depth (Fig. 5) shows marked deviations, at 3.70 m and above 1.90 m, from the linearity which would be expected in a constant sedimentary regime. Between these anomalies, however, a classical linear profile implies a uniform sedimentation rate, estimated at 0.31 mm yr⁻¹ by regression analysis, between 1,700 and 6,300 radiocarbon yr BP. Interpolation within this section dates the *Ulmus* decline at around 5,300 yr BP and the end of the marine incursion at about 5,450 yr BP. The anomalous datum point at 3.70 m is considered erroneous because of inwash of 'old' carbon-containing material, at the beginning of the marine transgression, such as would be derived either from erosion of the extensive body of late glacial silts which existed in the basin or from marine input of aged carbon. This view is supported first by the enhanced organic carbon levels in the sample (5% compared to the 1.5% norm) and second by the erroneously old ¹⁴C ages observed in the pre-industrial marine sediments of the adjacent Clyde Sea Area²⁰ through natural fossil carbon sources in the marine and freshwater catchment areas. For timescale evaluation, therefore, it seems most reasonable to interpolate directly between the 3.30 and 4.40 m age data, yielding a 6,640 yr BP age for the *Alnus* rise and about 6,900 yr BP for the onset of marine incursion.

Table 1 Palaeomagnetic dates on core LLRD1

Depth (m)	Feature			Age (yr BP)
0.20	a	D	W	150 o
0.30	α	I	H	250 o
0.45	b	D	E	450 a
0.60	c	D	W	600 a
0.70	β	I	L	650 a
0.95	d	D	E	1200 w
1.00	γ	I	H	1150 a
1.25	δ	I	L	1650 a
1.60	e	D	W	2400 w
1.90	f	D	E	3400 w
2.80	g	D	W	4500 w
3.60	h	D	E	6200 w
4.20	i	D	W	7400 w

D, declination: W, West; E, East.

I, inclination: H, maximum; L, minimum.

Age: o, magnetic observatory dates; a, archaeomagnetic dates; w, Windermere radiocarbon dates.

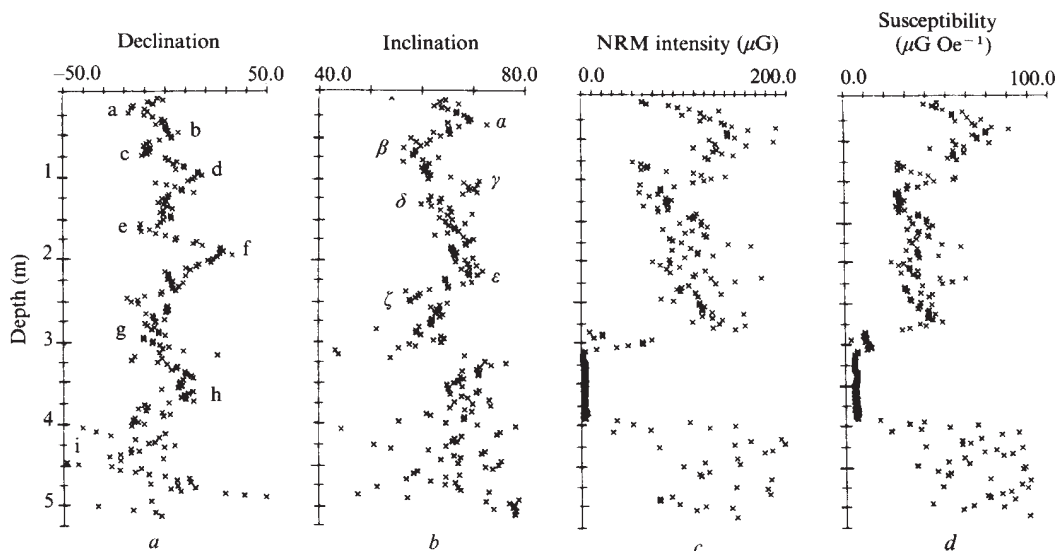


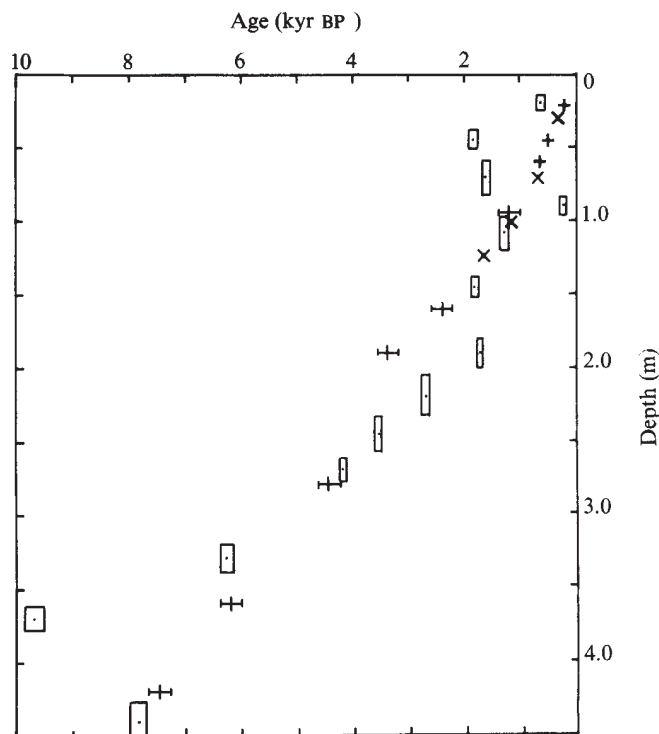
Fig. 4 Palaeomagnetic measurements. The ages of turning points (a-i) in declination and (α - δ) in inclination are shown in Table 1 and plotted as upright and diagonal crosses in Fig. 5.

The uppermost sediment layers have clearly been influenced by human activities such as forest clearance, agriculture and pollution. Thus quasi-vertical or negative gradient trends in ^{14}C age with decreasing depth are well-documented and are considered to be a direct consequence of man-induced inputs of fossil or old carbon through modification of run-off and erosion patterns or direct disposal of fossil fuel waste products²¹⁻²⁵. It is, therefore, impossible to define a calendar timescale for the upper 1.50 m of the sediment. The only confident implication is that an agriculturally-related perturbation became highly significant around 1,700–1,800 yr BP. At this time the first detectable anthropogenic depletion of ^{14}C levels in the sediment is paralleled by the rapid increase in grass pollen at 1.60 m (to values up to 75% AP). The younger ^{14}C ages of the 0.90 m and 0.20 m samples could reflect the effect either of temporary restabilisation of sediment deposition or of vertical mixing of nuclear-era artificial ^{14}C . The former possibility seems much more likely. Thus the 0.90 m age would be realistic, implying that a subsequent very rapid accumulation of sediment occurred either through increased catchment erosion or sediment slumping. This hypothesis is tentatively supported by analyses of metals by A. B. MacKenzie (personal communication). Concentrations of Al and Mn apparently show similar characteristic trends about a turning point at 0.90 m, suggesting bulk sediment input of old material above that depth. Thus it seems most probable at present that extremely rapid deposition of sediment commenced around AD 1700 and was subsequently followed by a more natural sedimentation pattern characterised by the surface pollen record and by ^{210}Pb dating. The latter was performed by Swan²⁶ on the top 15 cm of a mini-Mackereth LLRP core and indicates a mean surface sedimentation rate of about 1 mm yr^{-1} . This result is confirmed by radiocaesium analyses which show that the nuclear fallout of the early 1960s is contained within the top 2 cm of sediment. Curiously, when corrected for sediment compaction on burial, the present net sediment accumulation rate equates almost exactly to the 0.31 mm yr^{-1} value found by radiocarbon dating for the deeper sections of the core. This in turn confirms that, although the present pattern of sedimentation is close to natural, there have been periods of massive accumulation during the past two millennia, the first shortly after 1,800 yr BP and a second in the past three centuries.

It is interesting to note that, if palaeomagnetic analysis could be regarded as error-free, comparison of Loch Lomond ^{14}C and palaeomagnetic profiles below 1.50 m becomes essentially an exercise in intercalibration of Loch Lomond and Lake Windermere¹² radiocarbon timescales. Within the limitations imposed by this assumption and by the open physicochemical nature of organic sedimentary materials, agreement is reasonable. Since the major chemical contaminant at depth involves 'old' carbon, it is likely that, where significant deviations occur

between two ^{14}C profiles in related sediments, the younger timescale is more appropriate. Thus it is possible that between 2.70 and 1.50 m the Windermere-based palaeomagnetic timescale is discrepant because of the more marked early human alteration of the Lake District forest environment from 5,000 radiocarbon yr ago onwards²⁷. Conversely it is feasible that the Loch Lomond timescale below 2.70 m is wrong because of the contaminant effect of the marine incursion. The conclusion after such considerations must be that radiocarbon dating of lake sediments carries a considerably greater, but non-quantifiable, uncertainty than is implied by the statistical counting error term alone.

Fig. 5 Age plotted against depth for core LLRD1. Boxes represent radiocarbon dates, with widths corresponding to the $\pm 1\sigma$ error intervals (based on statistical counting uncertainties only) and heights corresponding to depth sampled. Upright and diagonal crosses define palaeomagnetic dates from observatory and archaeomagnetic declination and inclination records respectively. Horizontal bars represent correlations with Windermere radiocarbon dates at declination turning points and include $\pm 1\sigma$ radiocarbon counting errors.



Flandrian marine transgression in core LLRD1

The marine layer, darker in colour and higher in carbon content than the other sediments, is distinguished not only by the very low magnetic susceptibility and intensity (Fig. 4) but also by the presence of marine plankton and the absence of freshwater plants.

Cysts of planktonic dinoflagellates are exclusive to depth 3.05–3.75 m (Fig. 2). In addition to *Operculodinium centrocarpum* (Defl. and Cooks.) Wall, *Bitectatodinium tepikiense* Wilson and probably two species of the genus *Spiniferites* there is an abundance of *Lingulodinium machaerophorum* (Defl. and Cooks.) Wall, a species which indicates reduced salinity (<33‰) and shallow turbid conditions as in the eastern Irish Sea^{28,29}. Remains of freshwater macrophytes and algae such as *Littorella*, *Nuphar*, *Myriophyllum* and *Pediastrum* are poorly represented but spores of *Isoetes lacustris*, which is not known to grow in saline water, occur throughout the core except between 3.05 and 3.75 m (Fig. 2).

Molluscs related to the Flandrian transgression were recorded last century from Rossarden, near Luss²⁹. The species observed include *Mytilus edulis*, *Hydrobia ulvae*, *Macoma balthica* and *Littorina* sp. and were derived from a whitish clay with a 'strong peaty smell'. This shell bed 'should not be confused with the older arctic clay [Clyde Beds]' and is of a more recent character³⁰.

The single valve of the mollusc *Macoma calcarea*, found in the core at 3.30 m, had been bored by the predatory prosobranch *Natica* sp. Although commonly found in the Clyde Beds as at Lochgilphead³¹ and once recorded from clays at Inchlonaig, the northernmost large island in Loch Lomond³⁰, *M. calcarea* is now extinct in the British Isles. It has a high boreal distribution with an outlying Danish occurrence. The *Macoma* valve is most likely explained by derivation from the late Devensian clayey silts which were extensively eroded during the Flandrian transgression.

The marine transgression as judged from core LLRD1 began about 6,900 yr BP, some 1,400 yr later than the transgression in the upper Forth Valley³, where the sites, yielding the earliest radiocarbon ages, were outside the Loch Lomond re-advance moraines and hence in direct connection with the open sea. The time necessary to submerge the substantial barrier across the Vale of Leven may account for the dissimilarity. Loch Lomond remained salty for some 1,450 years, until about 5,450 yr BP.

As the saltwater influence declined, a period of meromixis can perhaps be envisaged; the fine lamination with anoxic conditions implied by the abundance of well-preserved pollen may support this speculation but detailed studies are required. Further work on pollen and macroscopic plant debris and especially on dinoflagellates in addition to diatoms and cladocera will help to elucidate the history of the loch.

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Nucleotide sequences at the ends of bacteriophage Mu DNA

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The nucleotide sequences were analysed at the two ends of bacteriophage Mu DNA. Such analyses reveal the existence of a short stretch of common sequences that are located at the termini and are orientated as inverted repeats. They also confirm that the heterogeneous bacterial DNA covalently bound to the ends of vegetative Mu DNA is totally removed during lysogenisation.

SINCE the pioneering work of McClintock¹, the existence of transposable elements that reversibly affect gene expression has been widely postulated (for a review, see ref. 2). In *Escherichia coli* these elements (IS, for insertion sequences) were shown to be transposed into various sites of the chromosome or episome, where they can promote deletions of

adjacent DNA. It has been noted that the ends of inserted IS are invariant^{3,4}, so that the recombination events involved in deletion formation and transposition are site specific. Mu is also capable of transposition, and again, the ends seem to be invariant, implying the presence of some special sequences at the termini of lysogenic Mu DNA. Because the vegetative and lysogenic maps are collinear⁵, these specific sequences must be near or at the ends of the DNA. In vegetative DNA these ends are covalently bound to bacterial DNA of various sizes and base composition^{6–8} and this heterogeneous DNA is presumably lost during the formation of a lysogen⁹.

Several laboratories are investigating the elucidation of the nucleotide sequences at the ends of various IS elements. It is hoped that this information will shed some light on the as yet poorly understood mechanism of IS transposition.

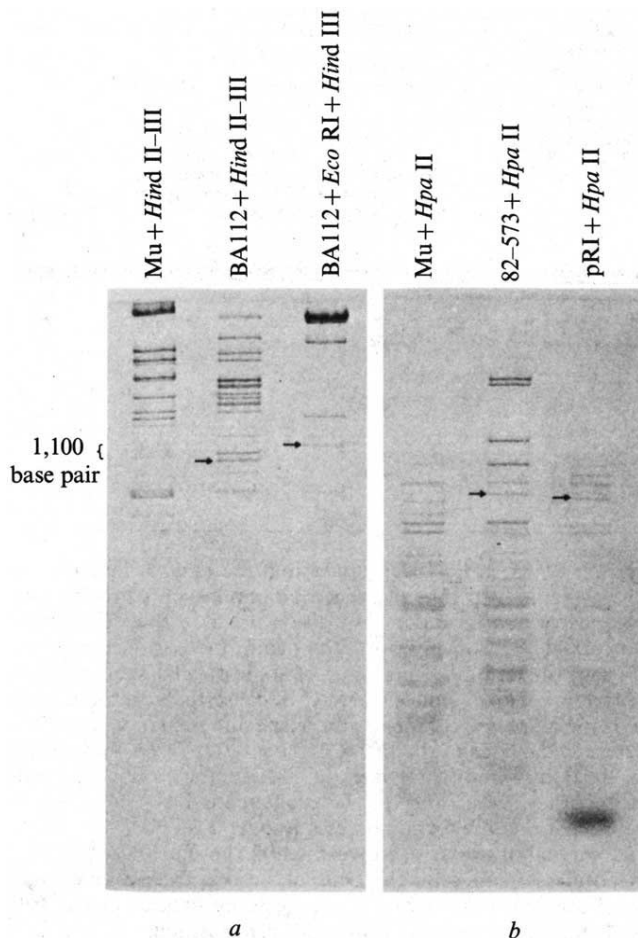
We report here an analysis of the nucleotide sequences at the ends of the Mu genome. Use was made of previously characterised hybrid particles in which Mu was inserted into the *lac Z* part of λ plac5 (refs 7, 10, 11). Our sequence assignments took advantage of the known amino acid sequence of the *lac Z* gene product¹². The results fail to reveal extensive sequence homologies at or near the two Mu ends. Rather, they pinpoint a short stretch of identical five base pairs that flank Mu and are orientated as an inverted repeat.

Our sequence analyses clearly show that the heterogeneous bacterial DNA is totally removed during lysogenisation. They also imply that identical Mu end sequences exist in lysogenic and vegetative DNA.

Sequences at the *c*-terminal end

Previous studies⁷ showed that a collection of λ pMu hybrids include the Mu *c*-terminal end (immunity end) in an *Hind*II + III fragment, smaller (1,000 base pairs) than the corresponding fragment of 1,100 base pairs cleaved from vegetative Mu DNA (Fig. 1a). Hence, it seemed that vegetative Mu DNA had sequences at the *c* end that were not present in the lysogen. As seen in Fig. 1a, the fragment from vegetative DNA formed a diffuse band in the gels. Judged by the breadth of this band, the heterogeneity apparently involved 50–100 base pairs. This assignment, with the migration data shown in Fig. 1a, dictated that an *Hind*II + III site must lie very close to the Mu *c* end in the λ pMu's. From subsequent work¹¹ we knew that this site

Fig. 1 Electrophoretic separation of DNA fragments containing one end of Mu. Results are shown for 2- μ g samples of the indicated digests run in a polyacrylamide gradient gel^{14,15} and stained with methylene blue. Fragments containing the *c* (a) or SE terminal end (b) are indicated by arrows. Note that in the Mu-*Hpa*II pattern the SE-end fragment forms a broad smear that cannot be seen in the gel.



was cleaved by *Hind*II and that it was located late in the *lac Z* gene. Figure 2a shows the Mu insertion site in a DNA fragment of 1,500 base pairs of λ plac5 and depicts the structure of BA112, a hybrid particle derived from the dilysogen and used in our study (Fig. 2b).

An approximate nucleotide sequence of the *lac Z* gene can be deduced from the known amino acid sequence of its protein product, β -galactosidase¹². We undertook to sequence the DNA that starts at the *Hind*II site in *lac* and extends into Mu. Divergence from the known *lac* sequences was expected to identify the sequences at the *c* end of the Mu lysogen. Purification of a fragment amenable to DNA sequence analysis by the method of Maxam and Gilbert¹³ was based on the cleavage data shown in Fig. 2c. Digestion of BA112 DNA by the mixture *Eco*RI + *Hind*III yielded a well resolved fragment of 1,120 base pairs that contained the Mu *c* end (Fig. 1a). This fragment was recovered from the gel, and a *Hind*II – *Hha*I fragment 700 labelled only at the *Hind*II site was prepared as described in Fig. 3. This fragment was treated with base-specific reagents according to the Maxam and Gilbert procedure. The products obtained are shown in Fig. 4. In good agreement with the prediction, the Mu sequences emerge early in the fragment, as seen in Fig. 5a. It should be noted that the dividing line between the *lac* and Mu sequences is somewhat arbitrary. Certainly, it could be one base pair to the left of the assigned site, as the *lac* sequence at that site is ambiguous, and it would start even farther to the left if the *lac* and Mu sequences happened to coincide for a few bases. Data from another independent insertion will be necessary to pin-point the *lac*–Mu junction unambiguously. This ambiguity, however, does not interfere with our main conclusion.

Sequences at the variable end

Starting with a dilysogen having Mu inserted in an orientation opposite to that seen in Fig. 2a, we could prepare λ pMu particles that contained the so-called variable or SE end^{6,9}. Again, Mu was inserted into *lac* DNA covered by the *Hind*II fragment of 1,500 base pairs (Fig. 2a), but at an unknown site. One of these particles, λ pMu 82–573 (ref. 11), was chosen for the present study. Preliminary experiments by hybridisation to appropriate DNA probes (labelled Mu fragments or λ pMu DNAs) led to the conclusion that the SE end was present in a well resolved *Hpa*II fragment of 770 base pairs (Fig. 1b). Because this fragment was not cleaved by *Hind*II (not shown) the *lac Hpa*II site must lie in the DNA covered by the *Hind*II fragment of 1,500 base pairs (Fig. 2a). To locate precisely this *Hpa*II site, we labelled *Hpa*II fragment 770 at the two ends as described in Fig. 3 and cleaved it with *Alu*I. Two one-end-labelled fragments of 320 and 60 base pairs resulted and were sequenced as in Fig. 4. Fragment 60 had no *lac* sequence homology and must contain only Mu DNA. The nucleotide sequence of fragment 320 was compatible with that of *lac* DNA starting at position 851 in the amino acid sequence of β -galactosidase, that is, 47 base pairs away from the leftward *Hind*II site of fragment 1,500 (Fig. 2a). No Mu sequences would be detected within the first ~100 nucleotides that could be sequenced in this experiment.

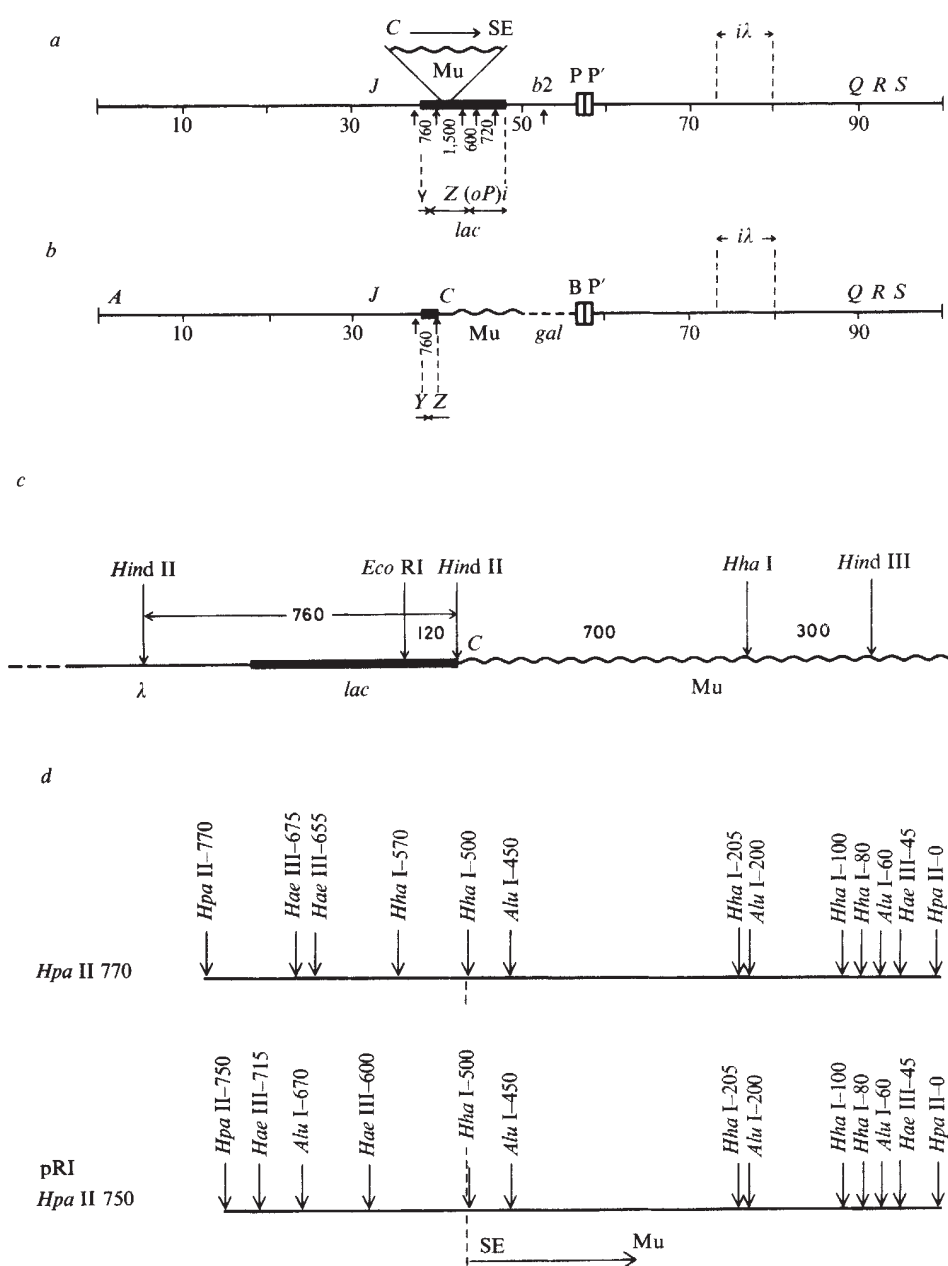
The following strategy was used to locate approximately the Mu insertion site in *lac* DNA. We constructed SE end-bearing plasmids by ligating a mixture of *Hind*III-cleaved pBR313 and a pure preparation of the heterogeneous *Hind*III fragment cleaved from vegetative Mu DNA and containing the SE end (not shown). This construction relied on the statistical occurrence of *Hind*III sites in the variable part of the Mu fragment. The ligated mixtures were used to transform *E. coli* K12 by standard techniques¹⁶ and the desired plasmids were selected by colony hybridisation¹⁷ to labelled probes containing DNA from the SE end. The plasmids selected in this way were purified and tested for retention of two *Hind*III sites. One of them (pR1) was chosen, as hybridisation data showed that it included the Mu SE end in a well resolved *Hpa*II fragment of 750 base pairs (Fig. 1b). It is clear that the only sequences this

fragment should share with the hybrid-derived *Hpa*II fragment of 770 base pairs must be Mu sequences. Moreover, these common sequences were expected to contain recognition sites for the same specific endonucleases, thus enabling us to locate approximately the Mu insertion site. To test this possibility we purified the plasmid fragment of 750 base pairs and the hybrid fragment of 770 base pairs, and compared them in their cleavage patterns with various restriction enzymes. The results of this analysis (not detailed here) are shown in Fig. 2*d*. From these data we assumed that the Mu sequences must emerge in the fragments somewhere between nucleotides 500 and 570 (measured from the right).

The validity of this prediction was verified by the sequencing analyses. Various restriction enzyme combinations were designed to sequence this part of the two fragments. An example is shown in Fig. 6*a*, where sequence was established starting

from the *Alu*I site at position 450 in the 82–573 hybrid DNA and extending to the left (Fig. 2*d*). A similar analysis was done starting from the *Hae*III site at position 600 in the plasmid DNA and extending to the right. The two sequences converged at nucleotide 505 in the fragments (that is, giving identical sequences to the right of the dotted line in Figs 2*d* or 5*b*, and different sequences to the left). Moreover, the sequences extending past this site in the 82–573 hybrid fragment were compatible with those deduced from the amino acid sequence of β -galactosidase starting at amino acid 755. This enabled us to deduce the nucleotide sequence at the SE end as seen in Fig. 5*b*. From the data obtained with the hybrid and plasmid DNAs the dividing line between the *lac* and Mu sequences can be drawn unambiguously. Figure 5*b* also includes sequences located on the right of the *Alu*I site at nucleotide 450 (Fig. 2*d*). They were obtained by labelling the ends of the *Alu*I fragment

Fig. 2 Physical maps. *a*, Mu insertion in λ plac5. The horizontal bar represents λ plac5 DNA calibrated in percentage units of molecular length measured from the left-hand end of the linear molecule. The heavy portion represents *lac* DNA whose genetic content is indicated under the bar. The vertical arrows point to *Hind*II sites, and the sizes of the fragments are expressed in numbers of base pairs. The Mu insertion is indicated above the bar where *c* stands for immunity end and SE for variable end. A, J, Q, R and S are λ genes; PP' represents the λ attachment site. *b*, Physical structure of λ pMu BA112. The representation is as in *a*. Note that the hybrid has arisen by abnormal λ excision and has the typical structure of λ *gal* particles near the attachment site. B stands for bacterial attachment site. *c*, Restriction enzyme analysis near the Mu insertion site in λ pMu BA112. The horizontal bar (λ), heavy bar (*lac*) and wavy bar (Mu) represent DNA in the vicinity of the Mu insertion site in λ pMu BA112 (*b*). The sizes of the fragments are expressed in numbers of base pairs. *d*, Cleavage patterns of two independent fragments including the SE-terminal end. *Hpa*II fragments 770 from λ pMu, 82–573 and 750 from the SE end-bearing plasmid (pRI) were eluted from a gel similar to that seen in Fig. 1*b*. Aliquots were end-labelled (Fig. 3) and submitted to either complete or partial digestion with the indicated restriction enzymes. The digests were electrophoresed as in Fig. 1 and the slabs were autoradiographed by contact with X-ray film. Labelled *Hae*III fragments of PM2 DNA were run in the same gel as size markers. The complete digests identified the terminal fragments and the size of the various partials permitted us to locate the different cleavage sites. A complementary analysis consisted of digesting to completion the cold fragments with various combinations of enzymes



and examining the sizes and numbers of the fragments thus obtained. The figure summarises these analyses. The numbers associated with the different sites represent the distance from the common end expressed in numbers of base pairs. The vertical dashed line indicates the starting site of the Mu sequences, as deduced from the sequencing data shown in Fig. 5*b*.

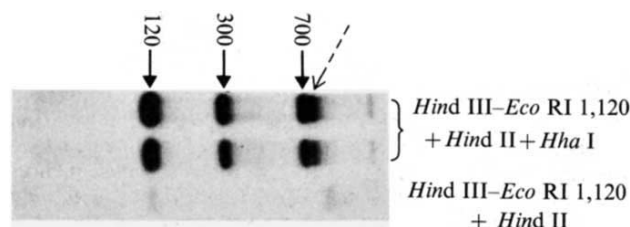
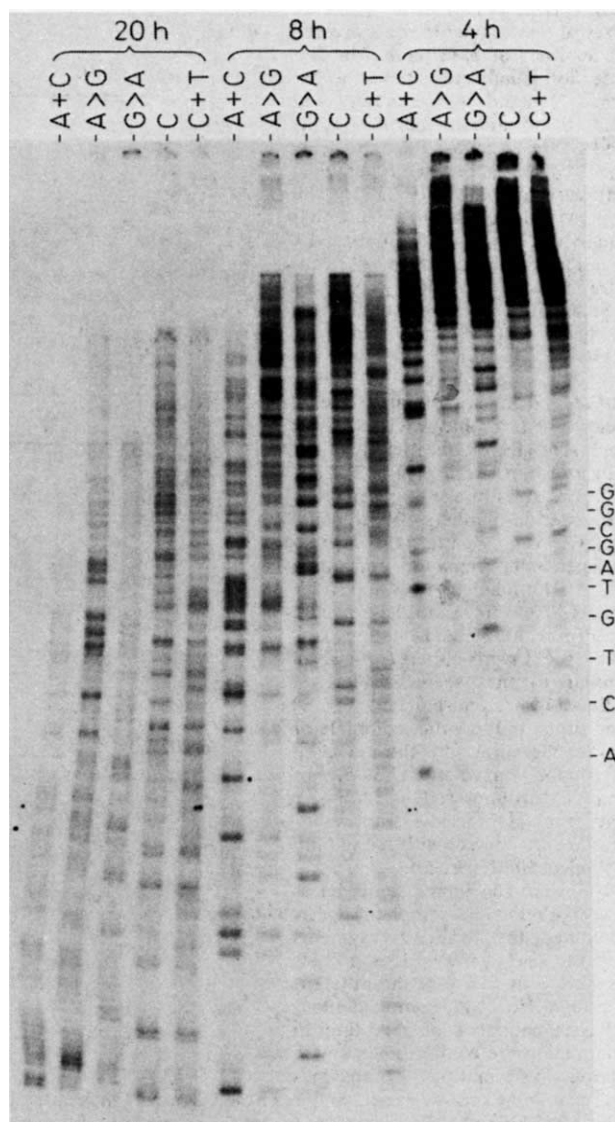


Fig. 3 Purification of *HindII-HhaI* fragment 700 labelled at the *HindII* site. 500 μ g of λ pMu BA112 were digested with the mixture *EcoRI-HindIII* in standard conditions, and the fragments were separated in a 2.5–7.5% polyacrylamide gel^{14,15}. The fragment of 1,120 base pairs (Figs 1a and 2c) was eluted electrophoretically into a dialysis bag, phenol extracted and concentrated by ethanol precipitation. The resuspended pellet was loaded on a Sephadex G75 column equilibrated in 10 mM Tris pH 8, 1 mM EDTA, and DNA-containing fractions were pooled and ethanol precipitated. The pellet was resuspended and digested with *HindII* in a 50- μ l reaction mixture. One unit of bacterial alkaline phosphatase (Worthington) was added 1 h before the end of the incubation. Reaction was stopped by addition of 2 μ l of 5% SDS and 5 μ l of 0.4 M EDTA; it was then phenol extracted three times. Phenol was removed with ether and the sample was ethanol precipitated, redissolved in 0.3 M sodium acetate, ethanol precipitated, and the final pellet rinsed once in 99% ethanol. The mixture of two fragments (*EcoRI-HindII* 120 and *HindII-HindIII* 1,000) was treated with T4 polynucleotide kinase as described¹³ except that the reaction was carried out at pH 8 (Tris) and without previous denaturation of the fragments in a 30- μ l volume. Excess ATP was removed by filtration through Sephadex G75 and the high molecular weight radioactive fractions were pooled and concentrated by ethanol precipitation. The resulting pellet was resuspended, digested with *HhaI* in a 50- μ l reaction mixture, and the digest was electrophoresed in a 5% polyacrylamide gel in Tris-acetate buffer¹⁴. A control without *HhaI* digestion was included. Electrophoresis was for 2 h at 100 V and the gel was exposed to X-ray film for autoradiography. The three main bands of 700, 300 and 120 base pairs indicated contain the *HindII-HhaI* fragment labelled at the *HindII* end (Fig. 2c), the *HhaI-HindIII* fragment labelled at the *HindIII* end and the *EcoRI-HindII* fragment labelled at the two ends. The dashed arrow points to a fragment whose presence reflects incomplete reaction of the *HindII* enzyme.

Sequencing at the two ends of IS1 (refs 3, 4, 19, 20) revealed the existence of an inverted repeat extending with mismatches over 20–30 base pairs. The sequences at the Mu ends seem quite different from those of IS1, and an inverted repeat can be drawn for only five bases. In addition to this homology at the ends, several sequence features were noted. Figure 5b pinpoints two palindromes near the SE end, but their biological significance cannot yet be assessed. We are now purifying early Mu proteins to test if these particular sequences correspond to specific protein-binding sites. Moreover, there exists near the c end (Fig. 5a) a set of seven bases that resembles the Pribnow box seen in all the known RNA polymerase binding sites²⁰. As Mu transcription will be studied in greater detail we hope to learn whether or not this set belongs to a promoter site.

It is important that identical SE end sequences are found in the plasmid which derives from vegetative Mu and in the

Fig. 4 Sequencing gel of the *HindII-HhaI* fragment 700 labelled at the *HindII* end. The reaction conditions for partial degradations were as described by Maxam and Gilbert¹³. The products were layered on a 1.5 $\times$ 30 $\times$ 35 mm denaturing slab gel prepared as described¹³ except that it contained 18% polyacrylamide. Pre-electrophoresis was for 6 h at 1,000 V, and the samples were run at this voltage for the times indicated. The slab was autoradiographed by contact with Kodak X-ray film (no-screen).



extending from nucleotides 450 to 200; the labelled ends were disconnected by either cleavage with *HhaI* (Fig. 2d) or strand separation in a non-denaturing gel. Figure 6b shows the products obtained with the faster moving separated strand. They permit deduction of the sequences starting from the *AluI* site at position 450 (Fig. 2d).

Discussion

Our ability to identify the Mu end sequences is based on the assumption that they are intact in the λ pMu hybrids analysed. The genetic material used and the mode of hybrid formation make it most likely that this is indeed the case. The original Mu- λ dilysogens have a single copy of Mu inserted into the *lac* part of λ plac5 (ref. 10). This copy is intact because plaque-forming Mu phages can be prepared from the strains. On the other hand, previous studies showed that λ pMu hybrids produced in these strains arose through a deletion starting in Mu and ending in λ . Hence, we are confident that the Mu end retained in these particles is intact and adjacent to the same genetic material as in the original dilysogen.

It has been reported that the insertion element IS1 is flanked by two sets of identical sequences orientated as direct repeats^{3,4}. Because the genetic material used to sequence the Mu ends was derived from different lysogens, we cannot know if Mu integration creates a similar duplication. Work is in progress to obtain particles containing either of the two ends from the same dilysogen. Such particles should also be useful to pin-point the *lac*-Mu junction unambiguously.

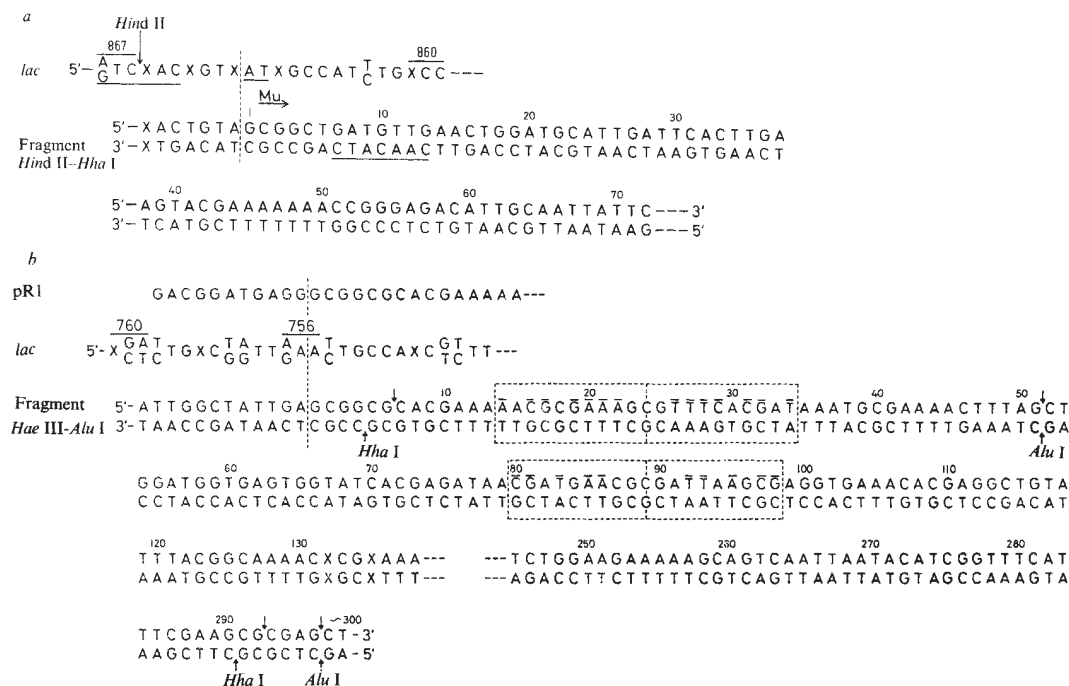


Fig. 5 Sequencing data. *a*, Nucleotide sequences at the C-terminal end. The nucleotide sequence is derived from the data in Fig. 4. The vertical dashed line indicates the site where the fragment sequences clearly diverge from the deduced *lac* sequences. Underlined is a set of seven base pairs which resembles a set found at a specific location in all the known *E. coli* RNA polymerase binding sites. The numbers above the *lac* sequences mark the positions in the amino acid sequences of the β -galactosidase protein¹². *b*, Nucleotide sequences at the SE end. The plasmid sequences and the deduced *lac* sequences are shown in the upper part. The vertical dashed line at amino acid 756 shows the site where the fragment sequences diverge from the *lac* sequences. The sequences in boxes have a rather high degree of palindromicity.

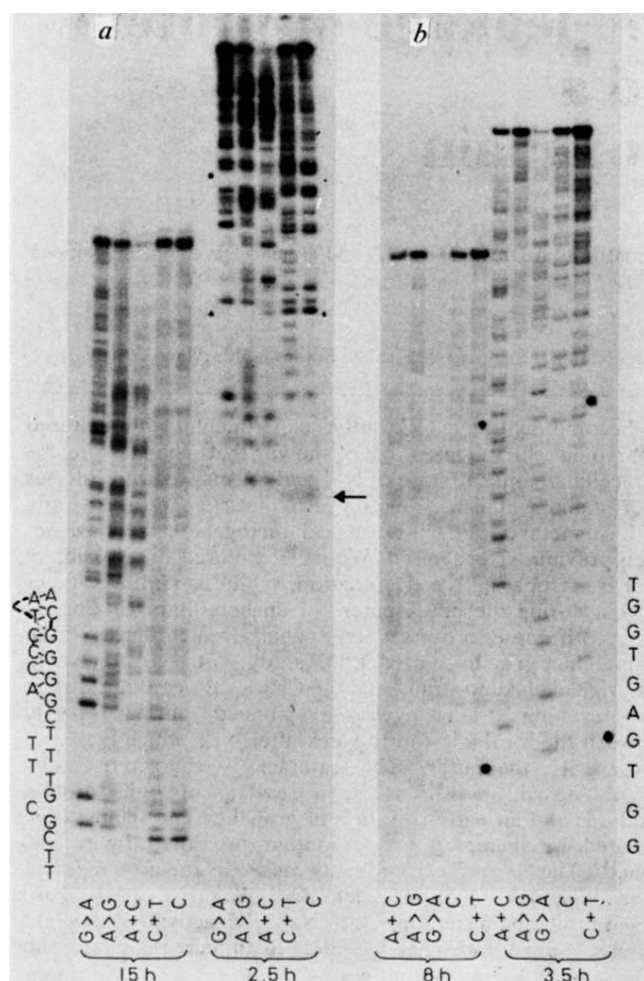


Fig. 6 Autoradiographs of sequencing gels. *a*, Fragment extending from *AluI*-450 to *HaeIII*-655 in λ pMu 82-573. 8 μ g of *HpaII* fragment 770 cleaved from λ pMu 82-573 (Fig. 1) were digested with *AluI*, treated with bacterial alkaline phosphatase, and ends labelled as described in Fig. 3. The digest was electrophoresed in a 5% polyacrylamide gel and the fragment of 320 base pairs extending from *AluI*-450 to *HpaII*-770 (Fig. 2d) was eluted from the gel and digested with *HaeIII*. This digest was submitted to polyacrylamide gel electrophoresis (5% gel) and the one-end-labelled fragment extending from *AluI*-450 (labelled end) to *HaeIII*-655 (cold end) was recovered and treated with base-specific reagents. The reaction and gel conditions were as in Fig. 4. The gel shown represents one-fifth aliquots of the total sample. They were designed to identify the early sequences near the *AluI* site and the sequences located 40-60 base pairs away from this site. Other aliquots (not shown here) were used for better resolution of other parts of the fragment. Sequences near the Mu-*lac* junction are marked on the left panel (note that the orientation is opposite to that shown in Fig. 5b). On the right panel (2.5-h run) the arrow points to a product that migrates with abnormal velocity. We interpret it as being free phosphate resulting from cleavage at the first nucleotide which is C (a consequence of the *AluI* specificity) and migrating much too slowly. *b*, Fragment extending from *AluI*-450 to *AluI*-200 in λ pMu 82-573. The *AluI* fragment of 250 base pairs labelled at both ends and extending from *AluI*-450 to *AluI*-200 (Fig. 2d) was eluted from the same gel as the fragment of 320 base pairs mentioned in *a*. Half of the sample was treated with *HhaI* to generate the one-end-labelled fragment extending from *AluI*-450 to *HhaI*-205 which was sequenced. The other half was used to prepare separated strands. It was dissolved in 50 μ l of 0.3 M NaOH, 10% glycerol, 1 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue¹³ and loaded on a 3-mm thick slab gel containing 7% acrylamide, 0.025% bisacrylamide, 50 mM Tris-borate, pH 8.3, and 1 mM EDTA. Electrophoresis was at 150 V until the xylene cyanol dye had moved 10 cm in the gel. The slab was autoradiographed by contact with X-ray film. The two strands were eluted separately and sequenced. The sequence shown is that of the faster moving separated strand. It is identical to that of the *AluI*-*HhaI* fragment labelled at the *AluI* end, thus identifying the sequences that start at the *AluI* site in position 450.

hybrid which comes from lysogenic Mu. A similar correlation presumably exists also at the *c* end. However, we have not been able to demonstrate it because attempts to clone the vegetative Mu *c* end in a plasmid have not so far been successful. Another important implication of the results presented here is that the heterogeneous bacterial DNA bound to vegetative Mu DNA is completely removed during lysogenisation. This is deduced from the finding that the Mu sequences are immediately adjacent to the *lac* sequences at both the *c* and SE ends. This observation, which confirms and extends previous data obtained by electron microscopy⁹, clearly shows that Mu integration is a site-specific recombination event as far as Mu sequences are concerned.

I thank Christiane Berger for technical assistance. This work was supported by the Swiss NSF.

Note added in proof: Since this article was accepted for publication, two independent sequences of the Mu *c* end have revealed a rather complex situation. In one case this end was present in a plasmid (pKNOO1) derived from lysogenic Mu (ref. 21). In the other case, the vegetative Mu *c* end has been cloned in a plasmid (pL4) in a way similar to that described above for cloning of the vegetative SE terminal end. In both cases the 5 base pairs characteristic of Mu end sequences were found almost unaltered but were followed by sequences different from those found in BA 112, as shown below:

	1		24
BA 112	 GCGGCTGATGTTGAACCTGGATGC	ATTGATT...	
pKN001	... TTTTTCGCGGTCGGTTG	ATTGATT...	
pL4	.. ACGCGGCGCGATGT	ATTGATT	

These results may indicate that the sequences located between the specific set of 5 bases and Mu position 24 in Fig. 5b are not selectively retained and can vary from one Mu particle to another. Alternatively, the heterogenous bacterial sequences bound to the *c* end of vegetative Mu DNA may participate somehow in Mu integration and thus account for *c* end heterogeneity even in the lysogenic state. Clearly, more data will be needed before we can distinguish between these possibilities.

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Differential metabolism and leakage of protein in an inherited cataract and a normal lens cultured with ouabain

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Ocular lenses in Nakano mice showed marked changes in synthesis, degradation and leakage of protein during cataractogenesis. The cataract-associated changes included the differential lowering of crystallin synthesis, the cleavage of crystallin polypeptides to lower molecular weight forms and the leakage of crystallins from cultured lenses. Ouabain treatment of normal lenses induced these alterations, suggesting that changes in the intracellular levels of Na⁺ and K⁺ affect the anabolism and catabolism of protein during cataract formation.

CATARACTS, or lens opacities, are caused by many different factors, such as physical and chemical insults to the lens, senility, disease and genetic defects¹. Although the mechanism initiating cataracts has been difficult to establish, hydration², protein aggregation³⁻⁵ and phase separation of water-protein mixtures⁶ seem to have important roles.

Several different types of cataracts are associated with Na⁺ influx and K⁺ efflux². The elevated Na⁺ concentration is believed to initiate and/or augment opacity by causing the lens

to become hydrated. Recently, experiments with cultured embryonic chick lenses have shown that changes in the intracellular concentration of Na⁺ and K⁺ may lead to changes in crystallin synthesis⁷. This suggests that changes in Na⁺ and K⁺ have metabolic effects during cataractogenesis that have not been previously recognised. We have explored this possibility here by comparing the degradation, synthesis and leakage of protein during the development of an hereditary cataract in mice with these processes in ouabain-treated lenses from normal mice. The mice used in this study were a cross between the original Nakano strain⁸ and the Charles River albino strain. The resulting strain develop a 'pinhead' nuclear opacity between the third and fourth week after birth which increases in severity thereafter. The cataract is characterised by morphological aberrations⁹⁻¹¹, a slowing of bulk protein synthesis and an early cessation of growth¹², and seems to be initiated by increased water content promoted by a Na⁺ influx¹³. The Na⁺ influx is probably caused by the production of an endogenous inhibitor of lens Na⁺, K⁺-activated ATPase activity. Ouabain inhibits the Na⁺, K⁺-activated ATPase activity of eukaryotic cells¹⁴, leading to an increase in Na⁺ and a decrease in K⁺ in cultured lenses¹⁵⁻¹⁸.

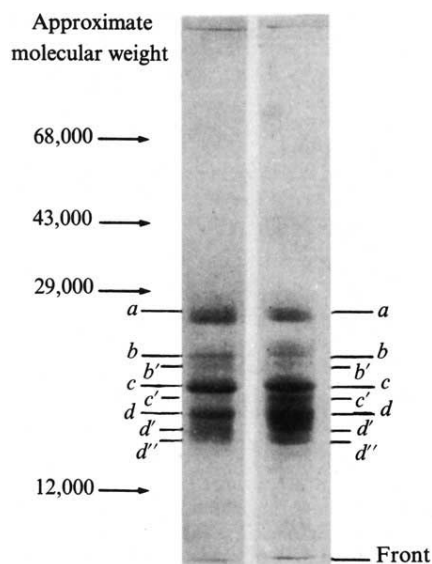


Fig. 1 Polypeptide compositions of 75-d-old normal (left) and Nakano (right) lenses. Individual lenses were homogenised in 2 ml of 2% SDS, 2% β -mercaptoethanol, 8 M urea (Schwarz/Mann, ultrapure), 10% glycerol, 1.2% Tris-HCl, pH 6.8 and a trace of phenol red, and heated to 100 °C for 3 min. Approximately 10 μ g of protein was analysed by discontinuous electrophoresis. Protein concentration was estimated by the values presented elsewhere¹². Electrophoresis was carried out in a 1-mm thick 10% polyacrylamide gel slab containing 0.1% SDS and 8 M urea at pH 8.8; a 4% polyacrylamide stacking gel in SDS and urea at pH 6.8 overlay the separating gel. Electrophoresis was at room temperature at 110 V until the phenol red reached the bottom of the gel, which took approximately 4 h. The gel was stained with 0.01% Coomassie brilliant blue R in 50% methanol and 50% acetic acid and destained by diffusion in 5% methanol and 5% acetic acid. Molecular weights were obtained by electrophoresis of marker proteins on a parallel gel: 68,000, bovine serum albumin; 54,000, leucine aminopeptidase; 43,000, ovalbumin; 29,000, carbonic anhydrase; 12,000, cytochrome c. See text for explanation of lettering. Note that bands c' and d'' are not present in the normal lens. Band a was resolved into two and sometimes three bands in different experiments.

Our findings indicate that lenses treated with ouabain strikingly mimic the cataractous lens in at least three parameters of protein metabolism: differential proteolysis, differential reduction of crystallin synthesis and differential leakage of proteins from the cultured lens. This suggests that ouabain-treated lenses in culture can serve as a useful model for the study of protein metabolism in this type of cataract.

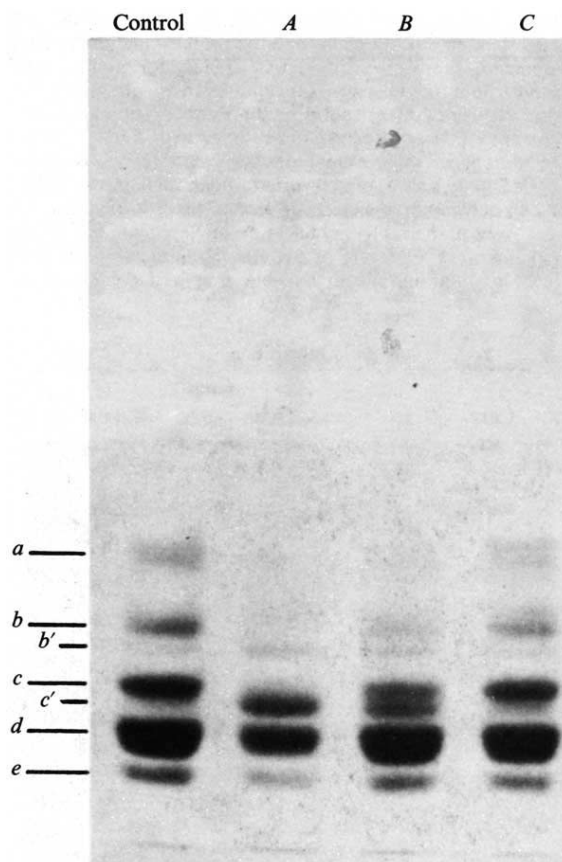
Appearance and polypeptide composition of normal, Nakano and ouabain-treated lenses

The lenses removed from normal (BALB/c) mice were transparent at all stages of development and remained clear when cultured for 24 h in modified¹⁹ TC-199 medium at 37 °C and in a 5% CO₂-95% air mixture. Lenses removed from Nakano mice were always transparent at 13 d after birth, but had pinhead opacities at 30 d and mature nuclear opacities at 70 d after birth. The cortical regions of Nakano lenses were always clear.

Ouabain treatment caused cultured lenses from normal mice to cloud and swell. All the ouabain experiments were carried out on 13-d-old lenses because of the greater biosynthetic activity at this stage. At 2×10^{-3} M to 1×10^{-4} M ouabain the lenses were swollen, diffusely opaque, and often contained pinhead opacities after 24 h of culture. At 2×10^{-5} M ouabain the cultured lenses were much clearer than at the higher concentrations and developed smaller pinhead opacities after 24 h *in vitro*. At 2×10^{-6} M ouabain the lenses remained clear for the 24 h of culture and did not seem to be swollen.

The polypeptides present in the normal and the Nakano mouse lens were examined by discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) and urea, a method which fractionates polypeptides on the basis of their molecular weight. There were several differences between the polypeptide compositions of 75-d-old lenses of normal and Nakano mice. Most striking was the appearance of bands c' and d'' in the Nakano lens preparation. In addition, band d was broader and band d' was more pronounced in the Nakano than in the normal lens preparation. A broad band between d' and d'' appeared only in the normal lens preparation. The Nakano lens preparation had a series of faintly staining bands between d'' and the front which were not apparent in the preparation from the normal lens. These were not present in sufficient concentrations to be easily visible in Fig. 1. Often, the front stained more intensely in Nakano lens preparations than in preparations from the normal lens, although this was not especially marked in the experiment shown in Fig. 1. The differences in the pattern of lens polypeptides between the normal and Nakano lens became detectable when the lenses developed pinhead opacities at approximately 30 d after birth. The electrophoretic patterns of lens proteins were the same in the clear lenses of 13-d-old normal and Nakano mice. Similar results were obtained in six different experiments.

Fig. 2 Effect of ouabain treatment on the polypeptide composition of 13-d-old normal lenses. Two lenses were cultured for 24 h in 0.7 ml of TC-199 medium alone made in bicarbonate buffer and 30 mM fructose, as described elsewhere¹⁹, or in the same medium supplemented with 2×10^{-3} M (A), 2×10^{-4} M (B) or 2×10^{-5} M (C) ouabain. Incubation was carried out in plastic dishes containing 16-mm diameter wells (tissue culture cluster dishes, Costar) at 37 °C in a humidified environment of 5% CO₂ and 95% air. The lenses were homogenised in 1.0 ml of electrophoresis buffer, subjected to electrophoresis and stained as given in Fig. 1. See text for explanation of the lettering.



The addition of ouabain to normal 13-d-old lenses resulted in marked alterations in the electrophoretic pattern of the proteins (Fig. 2). As the concentration of ouabain was increased, the intensity of bands *a*, *b* and *c* decreased and bands *b'* and *c'* became apparent. At the highest concentration of ouabain (2×10^{-3} M), there was also reduction in the staining intensity of bands *d* and *e*. Band *e* was gradually lost from the normal lens during maturation and had a faster electrophoretic mobility than band *d''* of Fig. 1.

To aid the identification of the bands and to test if bands *b'* and *c'* represented cleavage products of bands *b* and *c*, respectively, pulse-chase experiments were carried out with 35 S-methionine, and the proteins were immunoprecipitated before electrophoresis with antibodies prepared against α and β crystallin. The striking disappearance of bands *a*, *b* and *c* and the appearance of bands *b'* and *c'* were clearly evident in the ouabain-treated lenses (Fig. 3). Analysis of the radioactive immunoprecipitates indicated that band *a* represented at least two β -crystallin polypeptides, that band *c* represented an α -crystallin polypeptide, and that bands *b* and *d* represented mixtures of α - and β -crystallin polypeptides. Separate immunoprecipitation tests demonstrated that band *d* also contained γ -crystallin. In addition, these results suggested that bands *b'* and *c'* contained cleavage products of bands *b* and *c*, respectively, as they all immunoprecipitated with α -crystallin antibody. Moreover, the β -crystallin immunoprecipitate from the ouabain-treated lens preparation suggested that band *c'* also contained β -crystallin polypeptides. Further analysis of these bands is required to establish the nature of their components.

Fig. 3 Immunoprecipitation of proteins from 13-d-old control (Cont) and ouabain (Oua)-treated lenses. Two sets of two lenses were labelled with 500 μ Ci of 35 S-methionine (New England Nuclear, 542 Ci mmol $^{-1}$) for 5 h in culture dishes, as described in Fig. 2. One set of lenses was frozen at -20°C after the labelling period, and the other set was incubated for 24 h in nonradioactive medium containing 2×10^{-3} M ouabain. Both sets were homogenised in 0.4 ml of physiological saline; 50- μ l samples were removed for electrophoresis of the total proteins and the remaining homogenates were centrifuged at 10,000g for 5 min at 4°C . The supernatant fractions were precipitated overnight at 4°C with an equal volume of either calf α - or β -crystallin rabbit antiserum. The precipitates were washed three times with 0.01 M sodium phosphate, pH 6.4, before analysis. Electrophoresis was carried out as in Fig. 1. The gels were dried under vacuum on to filter paper and autoradiographed using Kodak SB-5 X-ray film. The antigens were prepared by gel filtration on Sephadex G-200; the β -crystallins were a mixture of β H and β L. Similar results were obtained in other tests with antisera prepared against mouse crystallins.

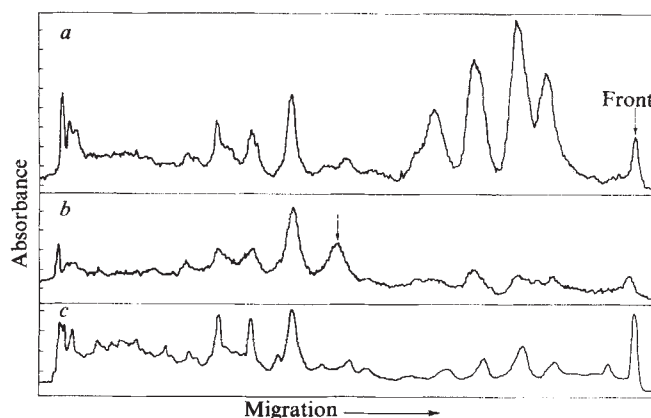
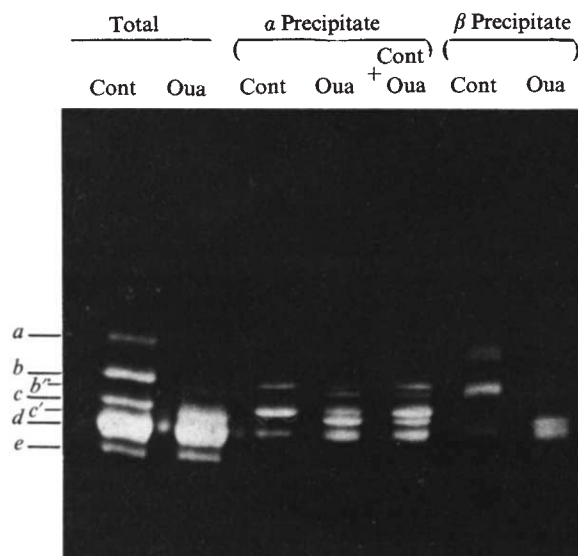


Fig. 4 Scans of autoradiograms of SDS-urea-polyacrylamide gels of proteins of 70-d-old normal (*a*) and Nakano (*b*) lenses, and of normal 13-d-old ouabain-treated lenses (*c*) after 5 h of labelling with 35 S-methionine as in Fig. 3. *a* and *b* were labelled immediately after explantation; *c* was precultured for 24 h in the presence of 2×10^{-3} M ouabain. Proteins were analysed by electrophoresis and autoradiography as in Fig. 3. Autoradiograms were scanned with a Quick Scan Jr densitometer. Approximately four times as much protein was examined from the Nakano lens as from the normal lens. This emphasises the extreme reduction in the synthesis of the crystallin polypeptides, which are located in the last third of the gel. The arrow denotes the protein band of the Nakano lens whose synthesis was least reduced.

Protein synthesis in normal, Nakano and ouabain-treated lenses

To test for qualitative changes in protein synthesis during cataractogenesis, we labelled normal and Nakano lenses from 70-d-old mice with 35 S-methionine for 5 h immediately after explantation, and examined the relative amount of incorporation into the different bands of lens proteins by electrophoresis and autoradiography of the polyacrylamide gel (Fig. 4). The Nakano lenses incorporated much less 35 S-methionine into protein than did the normal lenses. Therefore, approximately four times more protein was examined from the Nakano lens than from the normal lens. Comparison of Fig. 4*a* with 4*b* demonstrates that the 35 S-methionine incorporation into the crystallins (the more rapidly migrating proteins) was severely and differentially lowered in the cultured Nakano lens. The differential lowering of crystallin synthesis in the Nakano lens was not as pronounced at 25 or 40 d after birth as at 70 d.

Ouabain treatment of the normal lens also primarily affected crystallin synthesis. For these experiments normal 13-d-old lenses were cultured for 24 h in the presence of 2×10^{-3} M ouabain and then incubated for an additional 5 h with 35 S-methionine. The controls consisted of lenses treated similarly but without ouabain. Ouabain treatment inhibited incorporation of 35 S-methionine into protein by 75–90%. 35 S-Methionine incorporation into the crystallins was almost completely inhibited (Fig. 4*c*), whereas incorporation into the higher molecular weight proteins was much less severely affected. By contrast, the lenses cultured without ouabain incorporated the 35 S-methionine mostly into the crystallins, as shown in Fig. 3. Thus, the pattern of 35 S-methionine incorporation in the ouabain-treated lenses was similar to that in the Nakano lenses.

An additional experiment was carried out to investigate whether the preferential inhibition of synthesis of the crystallins was a characteristic of the cultured Nakano lens but not of the cataractous lens within the eye. Eyes were removed from 75-d-old normal and Nakano mice, and 225 μ Ci of 35 S-methionine contained in 1 μ l was injected into the aqueous humour with a sharpened Hamilton syringe. The eyes were incubated at 37°C in a 5% CO_2 –95% air mixture in a moist chamber. After 5 h the lenses were removed and the proteins

examined by SDS-urea-polyacrylamide gel electrophoresis and autoradiography. The autoradiograms (not shown) demonstrated that the incorporation of ^{35}S -methionine into the crystallins was differentially lowered in the Nakano lens, indicating that this phenomenon is not confined to the cultured Nakano lens.

Leakage of proteins from normal, Nakano and ouabain-treated lenses

We assayed the medium of cultured normal, Nakano and ouabain-treated lenses for the presence of proteins. Normal and Nakano lenses from 70-d-old mice were labelled with ^{35}S -methionine for 5 h, transferred to nonradioactive medium and incubated for another 24 h. Aliquots of the media were examined by SDS-urea-polyacrylamide gel electrophoresis. The staining patterns of electropherograms showed that very little protein was present in the medium of the normal lenses, but that considerable amounts accumulated in the medium of the Nakano lenses (Fig. 5). Leakage of protein into the medium was not evident in 13-d-old Nakano lenses, but did occur in 30-d-old lenses which had pinhead opacities. The polypeptides which preferentially leaked from the cultured Nakano lenses were β - and γ -crystallins according to their electrophoretic migration. The crystallins present in the medium were not radioactively labelled because, as demonstrated above, there is very little crystallin synthesis in the Nakano lens. Surprisingly,

however, there were some newly synthesised polypeptides with higher molecular weights than the crystallin polypeptides which leaked from both the normal and the Nakano lenses.

The medium of normal 13-d-old lenses cultured with 2×10^{-3} M ouabain also accumulated large quantities of protein. Leakage also occurred from lenses treated with 2×10^{-4} M ouabain. Most of this protein was γ -crystallin, as judged by its co-migration with purified γ -crystallin when subjected to SDS-urea-polyacrylamide gel. Band *e* and some β -crystallin polypeptides (principally band *a*) also leaked from the ouabain-treated lenses. Very little, if any, α -crystallin was present in the medium of the Nakano or ouabain-treated lenses. As with the 70-d-old lenses, autoradiograms showed that the cultured 13-d-old lens leaked newly synthesised, relatively large molecular weight polypeptides of high specific activity, irrespective of whether or not they were treated with ouabain (data not shown).

Discussion

Our present investigation demonstrates that ouabain-treated, normal mouse lenses mimic changes in protein degradation, synthesis and leakage which are shown here to be characteristic of the Nakano cataract. This is particularly interesting as both ouabain treatment of the normal lens¹⁵⁻¹⁸ and cataract formation in the Nakano lens^{2,13} result in an increase in the intracellular concentration of Na^+ and a decrease in the intracellular concentration of K^+ . Apparently, then, the use of ouabain-treated normal lenses offers an opportunity to study in controlled conditions alterations in protein metabolism similar to those which occur during cataractogenesis in the Nakano lens. It is not known, however, if the alteration in protein metabolism in the Nakano and ouabain-treated lenses is due directly to changes in the intracellular concentrations of Na^+ and K^+ , or whether it is a secondary consequence of other changes. Many cell-free experiments indicate that different concentrations of Na^+ and K^+ cause preferential translation of specific mRNAs (refs 20-26), leaving open the possibility that these cations have a direct effect on translation in the Nakano lens. One interesting possibility concerning protein degradation is that it is caused by the activity of a neutral endopeptidase which is associated with α -crystallin and apparently activated by hydration as Na^+ enters the lens^{27,28}. Further work is necessary to determine the causes of the changes in protein metabolism reported here and to establish that the changes in synthesis, degradation or leakage of protein in the Nakano lens operate by the same mechanisms as those in the normal lens treated with ouabain.

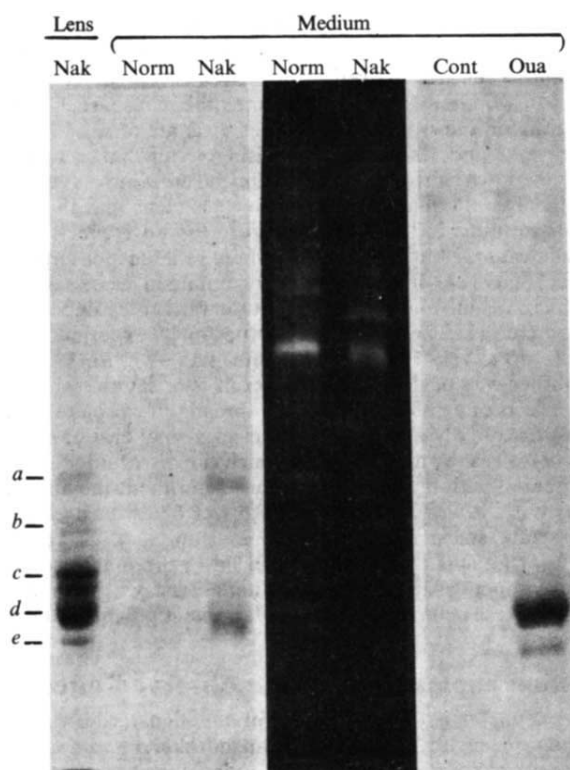
The present results indicate that changes in synthesis, degradation and leakage of protein may all contribute to the reduction in protein content of cataractous lenses²⁹⁻³⁹. Although our experiments did not quantitate the relative effects that the changes in synthesis, degradation and leakage would have on the protein content of a cataractous lens, the significant quantities of crystallin found in the medium of the cultured Nakano lens suggests that leakage may be a major cause of protein loss. It remains to be shown, however, that this leakage of crystallins from the Nakano lens also takes place *in vivo*. Immunochemical evidence for leakage of α -crystallin into the aqueous humour of humans with cortical, nuclear and complicated cataracts has been recently obtained⁴⁰.

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Fig. 5 Effects of cataractogenesis and of ouabain treatment on leakage of proteins from cultured lenses. Three 70-d-old normal (Norm) or Nakano (Nak) lenses and two 13-d-old control (Cont) or ouabain (Oua)-treated lenses were labelled with 570 μCi of ^{35}S -methionine (Amersham/Searle, 975 Ci mmol^{-1}) as in Fig. 3. After 5 h the lenses were rinsed once in physiological saline and incubated for another 24 h in 0.7 ml of modified TC-199 medium lacking ^{35}S -methionine. The following amounts were analysed by SDS-urea-polyacrylamide gel electrophoresis and autoradiography as in Fig. 3: 1% of one Nakano lens; 10 μl of the medium of the 70-d-old lenses; 20 μl of the medium of the control and ouabain-treated lenses. Total volumes of the media were 700 μl .



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A surprising property of electrical spread in the network of rods in the turtle's retina

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Flashing a localised stimulus onto a turtle's retina produces an intracellular potential wave which spreads through electrical connections from illuminated to unilluminated photoreceptors. The response in unilluminated rods (but not in cones) becomes faster as the distance from the source increases, perhaps because voltage-dependent permeability changes in the rod membrane make the coupled network behave like a high-pass filter.

MUCH evidence has accumulated to show that vertebrate photoreceptors in the peripheral retina are linked by direct electrical connections^{1–6}. These connections are specific in the sense that rods and cones of different spectral sensitivities form separate electrical networks (ref. 7 and P.B.D. and A.L.H., in preparation). A possible reason for electrical coupling is that it reduces the fluctuations resulting from the random absorption of light quanta by a factor of 10 or more^{2,4,6}. Such an improvement in the steadiness of the electrical signal is gained at the expense of a loss of spatial acuity⁶.

Measurement of electrical spread

The degree of coupling in the network of receptors was investigated by applying flashes of light through an optical system which illuminated a long narrow strip on the retina; this strip, which was 5 or 19 μm wide, was moved across a cone or rod impaled with a microelectrode in an eyecup preparation. The electrical signal recorded in the dark on either side of the strip decreased with distance in a roughly exponential manner. The length in which the potential fell to $1/e$ gave the space constant, λ , which is a useful measure of the extent of electrical spread.

In the network of red-sensitive cones in the turtles *Pseudemys*⁶ and *Chelydra*, λ was found to be 20–30 μm and in the rods of *Chelydra* it has been estimated⁴ as about 60 μm . On the assumption that the network acts like a low-pass filter with resistive connections and transverse R – C elements (Fig. 1a), one would expect the signal to reach a peak later as it is attenuated by moving the illuminated slit further from the impaled cell. Figure 2 shows that the network of red-sensitive

cones in *Pseudemys* behaved in this way and that the crest moved with a positive velocity of 2 mm s^{–1}. The mean velocity in 15 experiments was 2.7 mm s^{–1}, and a similar value was obtained in a red-sensitive cone in *Chelydra*.

It can be shown that if an electrical wave which is slow compared to the time constant, τ , of the membrane is attenuated by a continuous cable such as that in Fig. 1a, the peak of the wave travels at a velocity of $2\lambda/\tau$ where $\lambda = (R_M/R_S)^{1/2}$ and $\tau = C_M R_M$ (in which R_M , R_S and C_M are membrane resistance $\times$ unit area, sheet resistivity and membrane capacity per unit area, respectively). On this basis the mean velocity of 2.7 mm s^{–1} and λ of 26.5 μm give a τ of 19.6 ms which is about three times the usually accepted value for cones. We do not know whether the discrepancy implies a real defect of the theory or whether it arises from some other cause, such as light scattering or an error in the previous estimate of τ .

Figures 3 and 4 show the surprising result that in a similar experiment with the rods of *Chelydra* the crest moves earlier as the signal is attenuated by distance. It is clear that this effect cannot continue to times less than zero (because effects do not precede causes), but for the times where reliable measurements can be made we have consistently obtained crest velocities which are negative in the sense that the time to peak decreases as $|x|$ increases. The range of velocities in 15 experiments was from –140 to –590 $\mu\text{m s}^{-1}$ with a mean of –260 $\mu\text{m s}^{-1}$.

Another way of describing the result is to say that the electrical effects of an absorbed photon spread out over a relatively large area initially and then contract to a smaller area at long times. This is shown by Fig. 5 in which the distribution at 0.5 s is compared with that at 1.6 s. In this experiment the apparent space constant at 0.5 s after the flash was 67 μm , whereas the steady-state space constant obtained from $\int_0^\infty V(x, t) dt$ was 26 μm . ($V(x, t)$ is the response to a flash; there are technical difficulties in measuring the steady-state space constant directly owing to the extremely strong effect of steady lights in desensitising rods.)

Possible explanation of the observed effect

It is possible that the shortening of the signal with distance depends on negative feedback from horizontal cells or on a double set of connections between rods. However, there is no

clear evidence for either of these suggestions and we prefer an explanation along the following lines. It is assumed that rods, like cones, are linked together by ohmic resistances R_s , but that the rod membrane behaves like a circuit containing a large inductance, so that the rod network has some of the properties of a high-pass filter. The rod resting potential is about -40 mV and lies between a potassium equilibrium potential, V_K , of perhaps -80 mV and a sodium equilibrium potential, V_{Na} , of possibly $+40$ mV. If the sodium conductance of the membrane increases with a first order delay when the internal potential V is suddenly displaced in a negative direction, then, provided $V < V_{Na}$, it can be shown that for small displacements of V from the resting potential the impedance of sodium channels is equivalent to that shown for the transverse element in Fig. 1b (see refs 8 and 9). Here G_1 is the high frequency conductance and $(G_1 + G_2)$ is the low frequency conductance of the membrane; LG_2 is the time constant with which the sodium conductance approaches its steady-state value at the resting potential after a sudden displacement of voltage. A similar

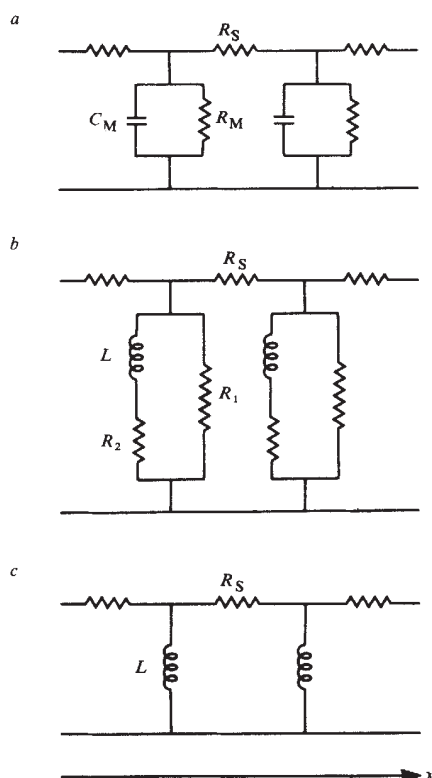


Fig. 1 One-dimensional models of receptor networks appropriate when the retina is stimulated by a strip of light at right angles to x . *a*, Model appropriate to network of red-sensitive cones in *Pseudemys*. R_s , sheet resistivity (Ω); C_M , membrane capacity per unit area; R_M , membrane resistance $\times$ unit area. If the network of red-sensitive cones is regarded as a square lattice with separation $17.4 \mu\text{m}$ between cones, then a single cone has a membrane resistance, r_m , of $\sim 200 \text{ M}\Omega$ and a capacity of 50 pF and the resistance, r_s , of a single link is $100 \text{ M}\Omega$ (see ref. 6 and P.B.D. and A.L.H., in preparation). *b*, Possible model for network of rods in *Chelydra*. R_s as above, L apparent inductance $\times$ unit area. $R_1 = 1/G_1$ and $R_2 = 1/G_2$ where G_1 is the high-frequency conductance per unit area and $(G_1 + G_2)$ is the low-frequency conductance per unit area. Assuming a square lattice with $20 \mu\text{m}$ between rods¹³, a d.c. input resistance¹³ of about $80 \text{ M}\Omega$ and a d.c. space constant of $26 \mu\text{m}$ (see text) approximate values, for a single rod, of $r_s = 3 \times 10^8 \Omega$ and $r_2 = 6 \times 10^8 \Omega$ are obtained. Values for the apparent inductance of $6 \times 10^8 \text{ H}$ and for r_1 of greater than $10^{10} \Omega$ reproduced satisfactorily the negative crest velocity and the space constant at early times (see Fig. 6). *c*, Simplified model of rod network.

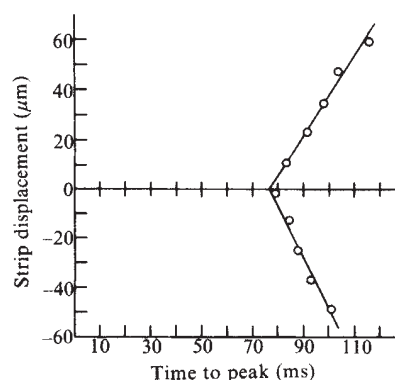


Fig. 2 Relation between time to peak of response to a 20-ms flash (abscissa) and distance (x) of impaled cone from centre of illuminated strip, which was $5 \mu\text{m}$ wide by 1.6 mm long. The peak response at $x = 0$ was 4 mV and at $|x| = 50 \mu\text{m}$ was 0.7 mV ; the maximum response to a strong large-field flash was 12 mV ; red-sensitive cone from *Pseudemys scripta elegans*; technique as in refs 6 and 7, wavelength 650 nm , flashes repeated at 0.5 s^{-1} .

equivalence holds if the potassium conductance of the membrane decreases with a delay for a negative-going displacement of V , provided that $V > V_K$.

Assuming that one or both of these mechanisms is present, and that both have the same time constant, it follows that we may use the circuit of Fig. 1b as a basis for calculating how electrical signals spread in the rod network. The rod response is so slow that it is probably legitimate to neglect the parallel capacity C_M . The curves in Fig. 6 were calculated on this basis and show that time-dependent conductance changes can account satisfactorily both for the negative crest velocity and for the change in waveform as the slit of light is moved away from the impaled cell. Evidence for the presence of an ionic mechanism which mimics an inductance is provided by the experiments of Copenhagen and Owen⁵ and Baylor and Fettiplace¹⁰, which show that rectangular pulses of current of either sign give overshoots in voltage when injected into the rod network. Such overshoots are not seen when small hyperpolarising currents are injected into cones in darkness (refs 1 and 10, and P.B.D. and A.L.H., in preparation) and this, together with the fact that the electrical response of cones is much faster than that of rods, may explain why cones show only the usual positive crest velocity expected from a low-pass filter.

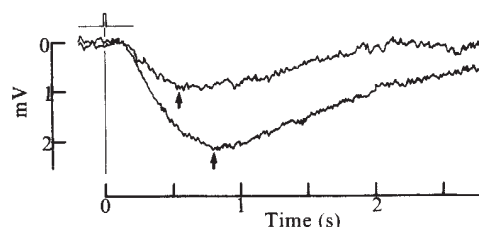


Fig. 3 Response of rod to a 20-ms flash which illuminated a strip of retina 1 mm by $19 \mu\text{m}$ at $18 \mu\text{m}$ (lower trace) and $77 \mu\text{m}$ (upper trace) from centre of impaled rod. Hyperpolarisation is shown downwards and the arrows mark the time of the peak hyperpolarisation. In the lower curve 22 responses were averaged, and 10 in the upper curve. The wavelength of the flash was 519 nm and the strength of the flash was $3.1 \text{ quanta per } \mu\text{m}^2$; flashes repeated at 0.05 s^{-1} . The maximum hyperpolarisation to a strong large-field flash was 25 mV and the sensitivity to a weak large-field flash was $9.1 \text{ mV per quantum per } \mu\text{m}^2$. Temperature about 20°C in this and other experiments. Rod from dark-adapted *Chelydra serpentina*, eye dissected under dim red light; electrode positioned using infrared light and infrared image converter; otherwise the technique was as in Fig. 2.

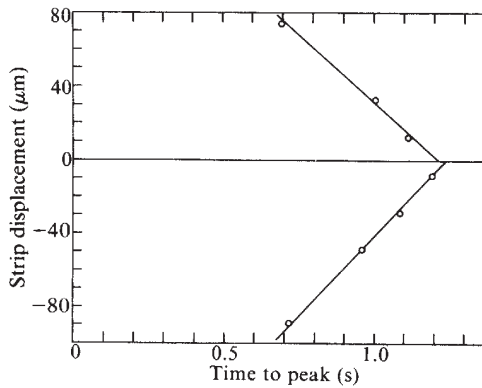


Fig. 4 Relation between time to peak of the response, on the abscissa, and displacement of the strip of light from the impaled rod. The intensity of the strip was 0.6 quanta per μm^2 and gave a peak response of 1.8 mV at $x = 0$ and 0.5 mV at $x = \pm 80 \mu\text{m}$. The maximum hyperpolarisation (V_{max}) to a large-field strong flash was 26 mV and the sensitivity to a dim flash was 20.4 mV per quantum per μm^2 . Other experimental details as in Fig. 3.

In the extreme case where $G_2 \gg G_1$ and the admittance $1/\omega L$ is roughly halfway between G_2 and G_1 , we have the simple case shown in Fig. 1c where the transverse element consists of an inductance L . The differential equation for this model is

$$\frac{\partial^3 V}{\partial x^2 \partial t} = \frac{VR_s}{L} \quad (1)$$

and if $V(0, t) = \exp(i\omega t)$, it follows that

$$V(x, t) = \exp(-x/\lambda_\omega) \cdot \exp(i\omega\{t - |x|/\theta\}) \quad (2)$$

where

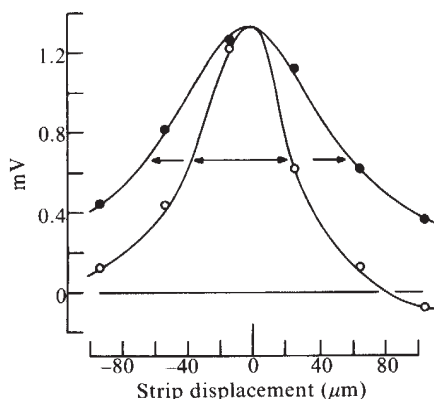
$$\theta = -\lambda_\omega \omega \quad (3)$$

and

$$\lambda_\omega = \left(\frac{2\omega L}{R_s}\right)^{1/2} \quad (4)$$

If $\lambda_\omega \doteq 60 \mu\text{m}$ and ω is taken as 4 s^{-1} , which is roughly the mean frequency component of the responses in Fig. 3, the velocity calculated from equation (3) is $\theta = -240 \mu\text{m s}^{-1}$, which is of the right order of magnitude.

Fig. 5 Distribution of potential at 0.5 s (●) and 1.6 s (○) after 20-ms flash, determined by plotting hyperpolarisation (upwards) against the displacement of the strip of light from the impaled rod. The arrows show the half-width of the distribution. Strip intensity was 1.3 quanta per μm^2 . V_{max} was 20 mV and the sensitivity was 11.5 mV per quantum per μm^2 .



Possible advantages of high-pass filter characteristics of the rod network

The high-pass filter characteristics of the rod network may be the result of an evolutionary adaptation which allows the effects of an absorbed quantum to be stored in a form in which bipolar cells can either integrate over distance to give good time resolution or integrate over time to give good spatial resolution. As the area over which current spreads from a single rod is proportional to λ^2 our results suggest that the patch of retina affected by an absorbed quantum should decrease by a factor of about 10, from a relatively large area at $t < 0.5 \text{ s}$ to a relatively small one after 2 s. Thus, a pathway which was specialised to respond with a short latency, or to pick out the high-frequency components of the rod response, would be affected by quanta collected over a large area, whereas a pathway with a long integration time would have better spatial acuity because the effects of each absorbed quantum become more localised with time. Such an arrangement would seem to have evolutionary advantages when objects are viewed in very dim light. Psychophysical effects, such as the variation of the critical frequency for fusion of flicker with the area illuminated¹¹, suggest that a similar phenomenon may be present in human vision.

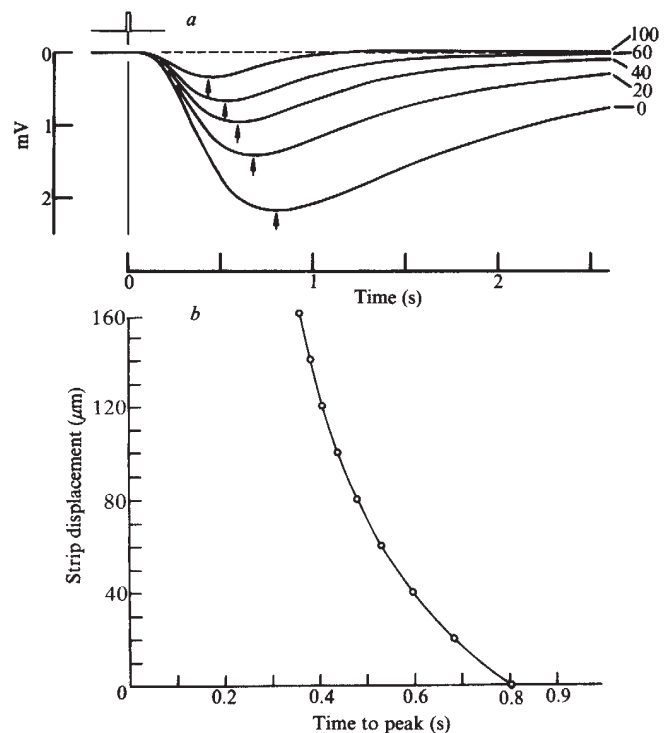


Fig. 6 The action of the network shown in Fig. 1b on rod responses. The finite-difference equation describing the network response was solved numerically for a 14-segment (that is, effectively infinite) cable, with the electrical constants and rod-spacing given in the legend to Fig. 1b; r_1 was taken as infinite. The voltage, in mV, directly under the illuminating slit was taken to be the following function, which is a good fit to the voltage recorded at $x = 18 \mu\text{m}$ in Fig. 3:

$$V = 4.35 (\exp(-0.135t) - \exp(-4.49t))^5$$

a, Voltages at various distances (shown in μm for each trace). Peaks are marked with an arrow. The space constant was $65.6 \mu\text{m}$ at 0.5 s, and $28.9 \mu\text{m}$ in the steady state. Note that the calculated response at $x = 100 \mu\text{m}$ swings positive after about 1.2 s, as was sometimes observed in experiments. *b*, Time to peak as a function of distance along the cable. The average velocity of the peak between $x = 0$ and $x = 60 \mu\text{m}$ is $-218 \mu\text{m s}^{-1}$.

The phenomena described here may help to explain another curious property of the rod network. From the experiments of Yau, Lamb and Baylor¹² it is clear that the current waveform injected into the rod network by the outer segments as a result of a strong flash of light consists of a rapid rise to a saturation plateau followed after a few seconds by an exponential decline to the dark level. If many rods are illuminated by a strong flash the internal potential recorded from the rod inner segment has a similar waveform except that the plateau of about -15 mV is preceded by a spike of -30 to -40 mV. This type of response would be expected if the current waveform observed by Yau *et al.* were injected into the transverse network of Fig. 1b. A similar phenomenon is seen in cones but is much less marked, the amplitude of spike and plateau being -25 and -20 mV, respectively.

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letters to nature

Gravitational radiation from supernova neutrino bursts

SUPERNOVAE, the violent collapses of massive stars into neutron stars, are considered to be the strongest known sources of gravitational radiation. The quadrupole (and higher) moments of the hydrodynamic motions of the infalling matter generate the radiation. A burst of neutrinos of total energy $\sim 0.1 M_{\odot} c^2$ and of duration between $\sim 10^{-3}$ and 1 s is thought to accompany the collapse¹. If this burst has nonspherical angular distribution, it will generate additional gravitational radiation—possibly comparable in magnitude to that caused by the collapse. That the neutrino burst might produce gravitational waves had been overlooked until recently, when Epstein suggested the idea². This problem is of interest in its own right because a massless field is acting as the source of a radiation field. In electromagnetism there are no charged, massless particles; in fact, the existence of such a particle would lead to infrared divergences³. The gravitational effects of neutrinos have been studied previously^{2,4–6}, but the approach we report here is new and has a nice analogue in electromagnetism. The emission of a neutrino is a quantum process and so we use the zero frequency limit (ZFL) quantum technique of Weinberg³ to obtain a classical source which gives correct results in this limit. Because the time scale of the burst ($\sim 10^{-3}$ –1 s) is much longer than that of the neutrino emission process ($\sim 10^{-22}$ s) only the ZFL is needed in the calculation. The ZFL technique has been used in an analogous way in electromagnetism to calculate the electromagnetic radiation accompanying β -decay or electron capture⁷. Smarr has applied the ZFL method to the gravitational bremsstrahlung process⁸.

Three problems arise in dealing with a superrelativistic source: first, the source is fast moving, here $v = c$. Second, the source moves with the radiation, so it is unbounded. And third, what is the correct expression for the stress-energy tensor to represent the creation of a neutrino? The first two problems render the usual quadrupole (or Landau-Lifshitz) formalism useless as they are applicable only to slow motion, weak field bounded sources. Instead, we retain only the weak field assumption and write the metric, $g_{\mu\nu}$, as $g_{\mu\nu} = \eta_{\mu\nu} + h_{\mu\nu}$, where $\eta_{\mu\nu}$ is the Minkowski metric and $h_{\mu\nu}$ contains the perturbations. Then in linearised gravity, in the Lorentz gauge ($\bar{h}^{\mu\nu}_{;\nu} = 0$), a wave equation can be written for $\bar{h}^{\mu\nu}$ ($\equiv h^{\mu\nu} - \frac{1}{2}\eta^{\mu\nu}h^{\alpha}_{\alpha}$),

$$\square \bar{h}^{\mu\nu} = -16\pi T^{\mu\nu} \quad (1)$$

where $G = c = 1$ and $T^{\mu\nu}(\mathbf{x}, t)$ is the matter stress energy

(including neutrinos). Equation (1) has the familiar solution in terms of a retarded Green function,

$$\bar{h}^{\mu\nu}(\mathbf{x}, t) = 4 \int T^{\mu\nu}(\mathbf{x}', t - |\mathbf{x} - \mathbf{x}'|) |\mathbf{x} - \mathbf{x}'|^{-1} d^3x' \quad (2)$$

To lowest order, both the matter emitting the neutrinos and the neutrinos travel on flat-space geodesics.

The energy carried off in gravitational waves can be found by computing the integral over a surface at infinity and over time of the gravitational energy flux given by the Landau-Lifshitz pseudotensor,

$$\delta E = \int \int t^0_{L-L} d^2S_i dt \quad (3)$$

The pseudotensor, t^0_{L-L} , is quadratic in $\bar{h}^{\mu\nu}_{,\lambda}$ so that only the parts of $\bar{h}^{\mu\nu}_{,\lambda}$ that vanish as r^{-1} as $r \rightarrow \infty$ contribute.

At first glance one might think that as the neutrinos created travel on flat-space geodesics they would not radiate, because a neutrino moving through flat space does not radiate. However, there is an important difference illustrated in Fig. 1. When computing the integral in equation (2) to obtain the contribution to $\bar{h}^{\mu\nu}(\mathbf{x}, t)$ from the free neutrino (in Fig. 1a), there is no term proportional to r^{-1} . But, when computing the same integral for Fig. 1b (neutrino created at $x = 0$), there is a term proportional to r^{-1} ; cancellations from the world line for $x < 0$ no longer take place because the neutrino did not exist there, and there is a r^{-1} contribution. The missing 'past history' of the created neutrino is the difference. The creation event is the key to the radiation.

This fact has two important consequences; first, the source is effectively local since once the neutrino is created, its future is determined because it travels (to lowest order) on a flat space geodesic. Second, the radiation is produced by a quantum process.

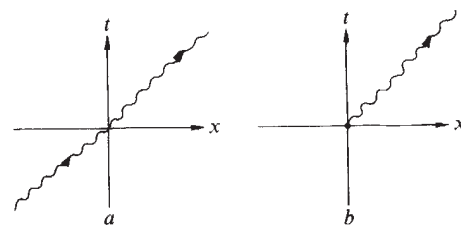


Fig. 1. A free neutrino propagating through Minkowski space from $x = -\infty$ to $x = \infty$ is shown in (a), and a neutrino created at $x = 0$ is shown in (b). The neutrino in (b) is lacking a 'past history', that is, it did not exist from $x = -\infty$ to $x = 0$.

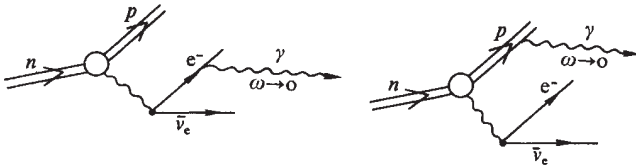


Fig. 2 Lowest order Feynman diagrams for the emission of a 'soft' photon during β -decay. In the limit $\omega \rightarrow 0$, these diagrams correspond to the classical current given in equation (5).

There is a similar process in electromagnetism: photon radiation from β -decay. Here, one classically expects radiation since two charged particles (an electron and a proton) must be 'accelerated from rest to their final velocities'. For the electromagnetic case the analogue of equation (2) is

$$A^\mu(\mathbf{x}, t) = \int j^\mu(\mathbf{x}', t - |\mathbf{x} - \mathbf{x}'|) |\mathbf{x} - \mathbf{x}'|^{-1} d^3x' \quad (4)$$

Naively, one might write for $j^\mu(\mathbf{x}, t)$

$$j^\mu(\mathbf{x}, t) = e\theta(t)[u_p^\mu/u_p^0\delta^3(\mathbf{x} - \mathbf{v}_p t) - u_e^\mu/u_e^0\delta^3(\mathbf{x} - \mathbf{v}_e t)] \quad (5)$$

where u_e^μ (u_p^μ) and $\mathbf{v}_e$ ($\mathbf{v}_p$) are the 4-velocity and ordinary velocity of the electron (proton). This classical current corresponds to two moving charges being created at $t=0$ and agrees with the quantum result (given by the Feynman diagrams in Fig. 2) in the limit of photons with zero frequency^{3,7}.

We have applied the ZFL technique of Weinberg³ to obtain a classical source $T^{\mu\nu}(\mathbf{x}, t)$ which is correct in the ZFL for the creation of a neutrino:

$$T^{\mu\nu}(\mathbf{x}, t) = \sum_{\text{in}} p_i^\mu p_i^\nu \delta^3(\mathbf{x} - \mathbf{v}_i t) \theta(-t) / p_i^0 + \sum_{\text{out}} p_i^\mu p_i^\nu \delta^3(\mathbf{x} - \mathbf{v}_i t) \theta(t) / p_i^0 \quad (6)$$

where $\sum_{\text{in}}$ ($\sum_{\text{out}}$) is the sum over in (out) going particles, p_i^μ are the particle 4-momenta and $\mathbf{v}_i$ are the ordinary velocities. The specific form of $T^{\mu\nu}$ depends on the neutrino production process. In the ZFL it is the changes in 4-momenta that produce radiation.

We adopt the following idealised model for the neutrino burst: (1) the neutrinos are created at $r=0$, travel on flat-space geodesics, and have an infinite mean free path, (2) the rate of creation $\sigma(t)$ has a time scale $\tau \sim 10^{-3}$ –1 s, and (3) the neutrinos have an angular distribution $f(\Omega)$. We can use linear superposition to write $\bar{h}^{\mu\nu}(\mathbf{x}, t)$ in terms of the contribution from one creation event in direction (θ, ϕ) , $\bar{h}_1^{\mu\nu}(\mathbf{x}, t, \Omega')$:

$$\bar{h}^{\mu\nu}(\mathbf{x}, t) = \int \int \bar{h}_1^{\mu\nu}(\mathbf{x}, t - t', \Omega') f(\Omega') \sigma(t') dt' d\Omega' \quad (7)$$

If we take the Fourier transform of $\partial/\partial t \bar{h}^{\mu\nu}(\mathbf{x}, t)$ (for convergence purposes), we find that

$$\partial/\partial t \bar{h}^{\mu\nu}(\mathbf{x}, \omega) = 2\pi \int (\partial/\partial t) \bar{h}_1^{\mu\nu}(\mathbf{x}, \omega, \Omega') \sigma(\omega) f(\Omega') d\Omega' \quad (8)$$

The quantity $\partial/\partial t \bar{h}_1^{\mu\nu}(\mathbf{x}, 0, \Omega')$ is given by the ZFL technique; $\partial/\partial t \bar{h}_1^{\mu\nu}(\mathbf{x}, \omega, \Omega')$ should have a width $\sim$ (time scale of creation process)⁻¹, which for e^- capture is $\sim 10^{22} \text{ s}^{-1}$. The width of $\sigma(\omega)$, τ^{-1} , is much less, and because of this only the ZFL of $\partial/\partial t \bar{h}_1^{\mu\nu}(\mathbf{x}, \omega, \Omega')$ is needed to very accurately compute

$\partial/\partial t \bar{h}^{\mu\nu}(\mathbf{x}, \omega)$ (see Fig. 3). The ZFL of $\partial/\partial t \bar{h}_1^{\mu\nu}(\mathbf{x}, \omega, \Omega')$ is given by $r^{-1} \exp(i\omega r) \partial/\partial t \bar{h}_1^{\mu\nu}(\mathbf{0}, 0, \Omega')$ so that

$$(\partial/\partial t) \bar{h}^{\mu\nu}(\mathbf{x}, \omega) = 2\pi/r \int \exp(i\omega r) \times (\partial/\partial t) \bar{h}_1^{\mu\nu}(\mathbf{0}, 0, \Omega') \sigma(\omega) f(\Omega') d\Omega' \bar{h}^{\mu\nu}(\mathbf{x}, t) = 2\pi/r \int \sigma(t') dt' \int (\partial/\partial t) \bar{h}_1^{\mu\nu}(\mathbf{0}, 0, \Omega') f(\Omega') d\Omega' \quad (9)$$

For a coherent burst of neutrinos only the ZFL of $\bar{h}_1^{\mu\nu}$ is necessary to compute $\bar{h}^{\mu\nu}(\mathbf{x}, t)$. The frequencies $\lesssim \tau^{-1}$ constructively interfere and dominate the wave form $\bar{h}^{\mu\nu}(\mathbf{x}, t)$.

For $f(\Omega)=1$, we find no radiation and vanishing Riemann tensor (at order r^{-1}). This is consistent with the exact results of Lindquist *et al.*⁵; however, without the proper $T^{\mu\nu}$ for the matter emitting the neutrinos, there is radiation and Riemann tensor at order r^{-1} . For $f(\Omega) \neq 1$, there is radiation and for similar matter anisotropies and hydrodynamic collapse time scales $\sim$ burst time scale, the energy released in gravitational waves produced by burst is equal to that produced by the hydrodynamic motions times a factor of the order of 0.001/(maximum matter velocity)⁴. These assumptions may overestimate the amount of burst produced gravitational waves in a real supernova.

Our model for the burst is highly idealised and subject to revision. To calculate waveforms processes must be chosen for neutrino production (for example, e^- capture and pair production). The question of mean free path must be considered more carefully.

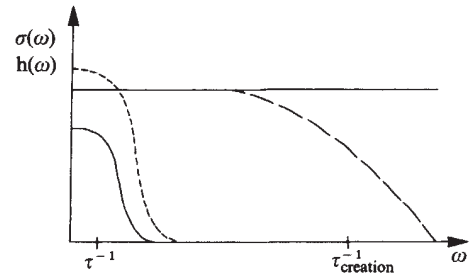


Fig. 3 The broken curve represents $|\partial/\partial t \bar{h}_1^{\mu\nu}(\omega)|$, the solid horizontal line $|\partial/\partial t \bar{h}_1^{\mu\nu}(0)|$, that is, ZFL approximation, and the dotted curve $\sigma(\omega)$. Note that $\tau^{-1} \ll \tau_{\text{creation}}^{-1} \equiv$ (time scale of neutrino production process)⁻¹ $\sim 10^{22} \text{ s}^{-1}$, so that to a good approximation $|\partial/\partial t \bar{h}^{\mu\nu}(\omega)| = |\partial/\partial t \bar{h}_1^{\mu\nu}(0)| \sigma(\omega)$ (shown as the solid curve). For simplicity the spatial agreements of $\bar{h}^{\mu\nu}$ have been suppressed here.

The process, radiation produced by a massless particle, is intriguing. The technique, use of the ZFL of a quantum process to obtain a classical source, is analogous to similar calculations in electromagnetism. The result that only the ZFL is needed to calculate the radiation from a coherent burst may be useful.

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Discovery of optical bursts from an X-ray burst source, MXB1735-44

THE detection of optical bursts¹ from the X-ray burst source MXB1735-44 (ref. 2) is reported here. Since the discovery of X-ray burst sources³, considerable efforts have been made to understand their nature. Recent reviews⁴⁻⁷ summarise the X-ray observations and proposed models which include accretion instabilities on to neutron stars or black holes and thermonuclear flashes on a neutron star (see also refs 8,9). As yet no final consensus of opinion exists about the burst mechanism or source geometry. Detection of bursts in other parts of the spectrum (radio, IR, or optical) could place significant limits on conditions at the source and provide additional tests of models. Thus an extensive campaign to monitor bursters was organised¹⁰ in 1977, and significant upper limits for emission (at burst times) in the radio^{11,12} and optical¹³⁻¹⁵ have already been reported. Our burst observations of the optical counterpart of 4U1735-44 (refs 16, 17), an intense persistent X-ray source associated with the burster MXB1735-44 (refs 2, 7), were made with considerably higher sensitivity than previously.

Optical monitoring of the stellar object identified with 4U1735-44 was conducted between 1 and 3 June 1978, with the 1.5 m telescope at Cerro Tololo Inter-American Observatory (CTIO). Simultaneous X-ray observations were made with the SAS 3 observatory, which maintained continuous pointing to keep 4U1735-44 centred within the 1.7° (FWHM) field of view of the horizontal tube detectors¹⁸. No bursts were detected by SAS 3 immediately before the 1 June observations, and so UBVP photometry of 4U1735-44 (and other objects) was conducted instead of continuous monitoring. X-ray burst activity commenced at 13:13 UT 1 June and therefore continuous optical monitoring as well as UBVP photometry were conducted from 02:15 to 10:30 UT 2 June and (though interrupted by clouds) from 02:40 to 06:00 UT 3 June 1978. Standard stars as well as the 4U1735-44 optical counterpart were measured in UBVP during the ~30 min periods in each SAS 3 orbit when 4U1735-44 was occulted (for SAS 3) by the Earth. These measurements indicated stable conditions, though with 25% increased extinction (due to thin haze), throughout the entire 2 June observations. The UBVP photometry on 1 and 2 June also indicated that the magnitude and colours of the 4U1735-44 optical counterpart were within 0.1 mag of each other and of the values measured previously¹⁶, although the source was known to be burst-active on 2 June but probably not on 1 June.

Every effort was made to obtain the maximum possible sensitivity for bursts: a high quantum efficiency GaAs RCA C31034 photomultiplier was used with a 10 arcs (diameter) circular diaphragm and a CuSO₄ filter to provide a broadband response peaked in the blue and well matched to the colours of the optical counterpart of 4U1735-44. The filter has 90% transmission from 3,500 to 4,800 Å and, combined with the photomultiplier response and atmospheric transmission, our effective bandpass was 3,100-5,500 Å (FWHM). The optical data were recorded on digital tape in 100 ms integrations and displayed in real time in 10 s integrations. Absolute time was maintained to within 10 ms of UT by continuous monitoring of WWV. Acquisition and guiding on the optical object were done using an integrating TV system; guiding adjustments were required approximately every 10 min.

The total count rate on the object was ~430 counts per second (c.p.s.) (for intermediate zenith angles) of which the sky background contributed ~260 c.p.s. Variations in the total rate displayed were very smooth and consistent with Poisson fluctuations about the typical value $4,300 \pm 66$ counts per 10 s. However, as then noted on two (and only two) occasions during the night the 10 s count totals departed significantly from the average. The first was at 06:49:18 UT 2 June 1978

when (during the previous 10 s) 4,862 counts were recorded, and the second was at 08:22:23 UT when 4,641 counts were recorded. Approximately one hour after this second event, the CTIO observers learned by telephone from MIT that an X-ray burst had been detected from MXB1735-44 beginning at 08:22:22, or within the second of the intervals noted. A few hours later we were able to retrieve the optical data from the tape and display the optical count rate in 1-s bins. The results for these two preselected optical events are shown in Fig. 1a and b, respectively. The burst appearance of both events is striking; within a ~1-2 s rise time the quiescent optical intensity of 4U1735-44 brightened by ~50% followed by a decline over the next ~7 s.

Optical burst 1 occurred while SAS 3 was dumping data to a ground station and so no X-ray data are available. SAS 3 data were recorded, as mentioned above, for optical burst 2 and are shown in two energy ranges in Fig. 1c (5-10 keV) and 1d (2.5-5 keV). The truncation of the X-ray data after burst 2 is due to another data dump. Figure 1b-d clearly establishes that the optical bursts are the counterparts of X-ray bursts. The burst profiles are qualitatively similar, and to within the accuracy of the SAS 3 quick look data timing (± 1 s), the optical burst onset is coincident with that in X-rays (SAS 3 production data not yet available will be significantly more accurate). The burst rise times are ≈ 2 s, and the major portion of the rise of burst 2 occurs in ≈ 1 s (searches for faster fluctuations are limited by the statistics). The optical burst duration seems to be ~9 s compared to ~2 s in the 8-18 keV band (not shown in Fig. 1), ~2 s in the 5-10 keV band and ~6 s in the 2.5-5 keV band. Most of the X-ray energy is in the two higher bands. The flux averaged over the burst increased by a factor of 8 for X rays and 1.25 for optical. Integrating over the burst duration, the ratio of total optical energy density (in the B filter bandpass) to the X-ray energy density (7.3×10^{-8} erg cm⁻²) for burst 2 is $\sim 2 \times 10^{-5}$ whereas the corresponding ratio for the steady emission is 1.3×10^{-4} . The X-ray energy density is derived from a flux calibration on the Crab nebula, while the optical energy density is obtained from the total (~400) number of optical photons detected in burst 2 and a calibration of our CuSO₄ filter with UBVP filter measurements on standard stars¹⁹. We estimate the combined statistical and systematic uncertainty in the detected burst optical energy density to be ~15%.

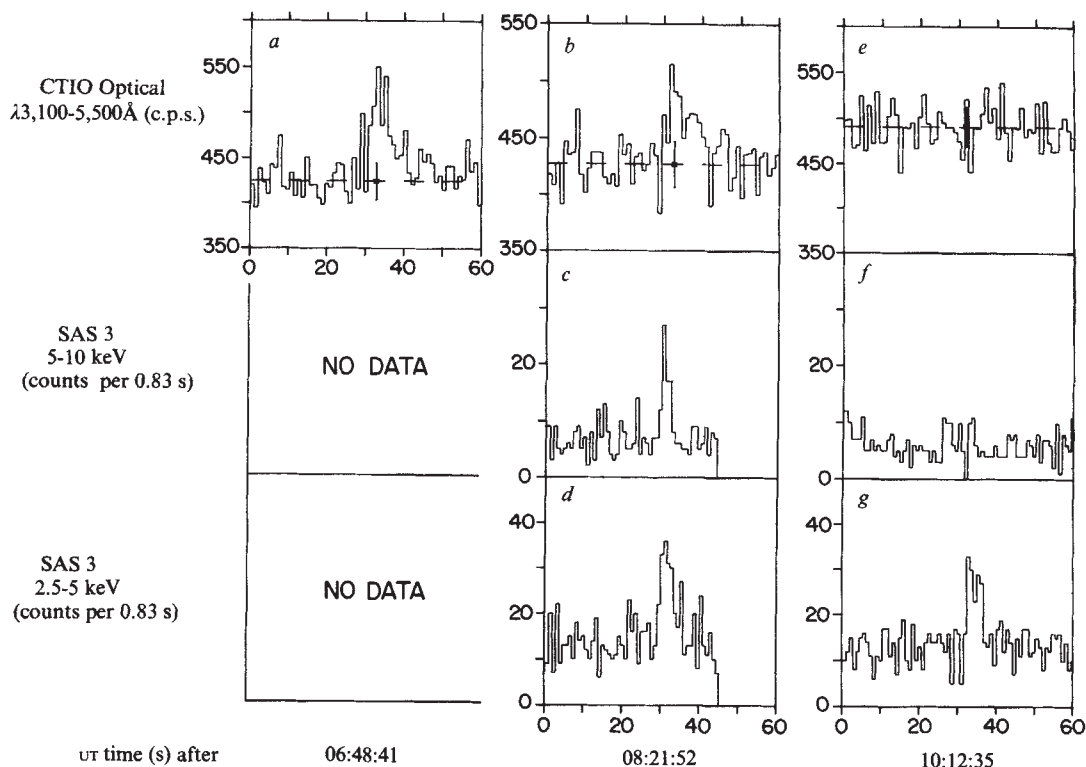
A third burst was detected by SAS 3 at 10:13:06 UT, which was ~7 min after the beginning of astronomical twilight at CTIO. The optical (with correspondingly larger background) and X-ray data for this event are plotted in Fig. 1e-f. Clearly no significant optical burst was detected. The X-ray data contain a 0.8 s gap (which may be corrected in the production data). Therefore, the total X-ray energy in burst 3 is $> 1.3 \times 10^{-8}$ erg cm⁻², or a factor of <5.6 smaller than burst 2. The soft (2.5-5 keV) fluxes are comparable. From the optical data, burst 3 is a factor of at least 2.7 (at the 90% confidence level) smaller than burst 2 when both are averaged over the first 3 s. The ratio of total optical to X-ray energy density integrated over burst 3 is, therefore, $< 4 \times 10^{-5}$, which may be consistent with burst 2.

The optical burst rise time and structure show that a significant portion of the optical burst must come from an emitting region which is within 1-2 light s of the X-ray source and ≈ 1 -2 light s in extent. It is also possible to derive the minimum size of the optical burst emitting region by equating the observed optical flux (corrected for the estimated extinction¹⁷ $A_v \approx 0.5$ mag) with that expected from a black body of temperature T . T must be $\approx 5 \times 10^6$ K for the X-ray flux from this region not to exceed the observed flux. The minimum radius for the optical burst emission region is then

$$R \geq 4.5 \times 10^8 (T/5 \times 10^6 \text{ K})^{-1/2} (D/10 \text{ kpc}) \text{ cm}$$

where D is the distance to the source. The burst optical emission region is therefore larger than the X-ray emission region if the X-ray is due to black-body emission, as has been

Fig. 1 Optical and X-Ray bursts from the X-ray burst source MXB1735-44. Optical data for three bursts are shown in: *a*, burst 1; *b*, burst 2; and *e*, burst 3 at the indicated times on UT 2 June 1978. No SAS 3 data were recorded for optical burst 1, but the X-ray data are shown for bursts 2 and 3. The average optical count rate on the source (which was ~ 170 c.p.s. above sky background) derived before the burst onset is shown in the optical burst plots as the dashed lines with $\pm 1\sigma$ error bars. The preburst average X-ray count rates (including the total background rate of ~ 4 counts per 0.83 s) in the (2.5-5, 5-10) keV energy ranges were (13.5, 5.6) and (12.2, 6.2) counts per 0.83 s for bursts 2 and 3, respectively.



indicated for several other burst sources and as would be expected for a thermonuclear flash model^{7,9}. A larger optical emission region could also account for the apparently longer optical burst duration.

The present results on optical bursts do not eliminate any of the various burst models. However, the optical observations do have implications for models involving a binary companion²². Namely, they indicate that all the optical emission probably does not arise from the X-ray heated stellar atmosphere of a possible companion. (The colours and spectrum¹⁷ of the steady emission also rule out a normal star.) The lack of variability (< 0.1 mag) of the steady optical source as noted in our data and in earlier observations^{16,23} also argues against significant optical emission from a heated binary companion.

Calculations of the effects of X-ray heating predict that the factor by which the optical flux increases (at the burst peak) should equal the corresponding factor for the X-ray increase raised to the power α . The predicted value of α is ≥ 0.25 for a hot black body atmosphere (as pointed out by P. Joss and S. Rappaport) and is ~ 0.3 to ~ 1 for several detailed models^{20,21}, whereas our observations give the value 0.22 ± 0.08 .

If the X-ray burster is in a binary system, the companion may be either shielded²⁴ from the X-ray source or unshielded but sufficiently separated from it so that its optical emission is small. In either case, as discussed above, the burst optical emission would arise from a region near the X-ray source. This could be the inner part of an accretion disk. The steady optical emission could arise from the outer part of the same disk or from a more extended region.

Further simultaneous X-ray and optical observations of optical bursts with still greater sensitivity and spectral resolution may provide stronger tests of burst models.

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An X-ray outburst from the RS CVn binary HR1099

RS CANUM VENATICORUM systems are the most plentiful binary stars known, having a space density of at least 10^{-6} systems pc^{-3} (ref. 1). They are detached subgiants of class KO+F to G with orbital periods between 1 day and 2 weeks (ref. 1). Many exhibit wave-like distortions in V which sometimes migrate to earlier binary phase with periods of 10–20 yr. This phenomenon has been interpreted by Hall² as resulting from extensive star spot activity on the surface of the cooler companion. Steady radio emission has been detected from several RS CVn systems with occasional flares involving an order of magnitude change in flux^{3–5}. More recently, HEAO-A has detected soft X-ray emission (0.2–2.8 keV) from three of these systems HR1099, UX Ari and RS CVn itself^{6,7}, with source luminosities of $\sim 10^{30}$ erg s^{-1} . We report here the detection by the Copernicus 2.5–7.5 keV detector of a 3-h outburst from HR1099, coincident with a radio flare.

In September 1976, the Princeton University ultraviolet spectrometer on board Copernicus was used in a coordinated UV, radio and visual study of HR1099 (ref. 5). This instrument is coaligned with the UCL/MSSL X-ray detector⁸ and during the vast majority of the UV observations this instrument collects background data⁹. Figure 1 shows the background subtracted flux recorded in 30-min time intervals during the first two days of the HR1099 observations and the two preceding observations of UX Ari and the X-ray target X Per (3U0352+30) (ref. 10). When Copernicus was pointed at X Per, a clear excess of counts above the background recorded during the UX Ari observation can be seen. The subsequent pointing at HR1099 revealed a 5σ signal, similar in strength to that recorded from X Per, for about 3 h centred on 0600 h on 24 September 1976.

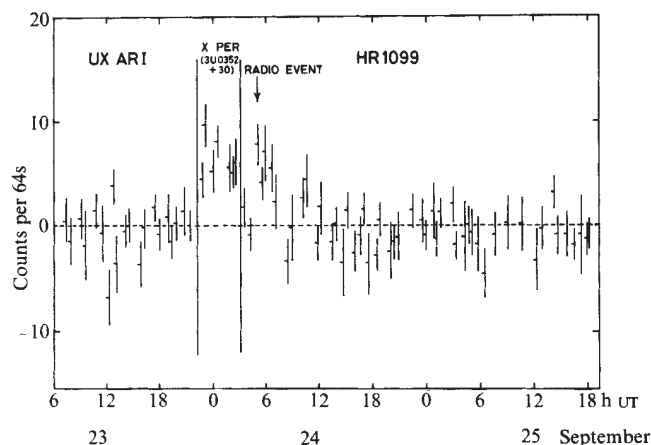


Fig. 1 The flux seen in the 2.5–7.5 keV band integrated in 30-min time bins. One count $\sim 4 \times 10^{-11}$ erg cm^{-2} s^{-1} .

This coincides with a radio outburst and enhanced $H\alpha$ and $Ly\alpha$ emission from HR 1099 (ref. 5), that lasted for less than a day. Only one radio measurement was made at 0500 h UT and hence no information is available about the hour by hour evolution of the radio event. The precise end of the X-ray event is unclear as there is some evidence at the 2σ confidence level for a second outburst 2–3 h after the principle flare-up. The flux is too low to obtain a spectrum; however, the total flux is very insensitive to the assumed spectral model. A power law photon index of -2.0 yields a source luminosity of $(2.2 \pm 0.4) \times 10^{31}$ erg s^{-1} in the 2.5–7.5 keV band, assuming a source distance of 33 pc (ref. 11). Observations of other background

targets revealed no evidence for a quiescent flux from either HR1099 or UX Ari at a level of 6×10^{-11} erg cm^{-2} s^{-1} (7×10^{30} erg s^{-1} for HR1099). There was a total of three weeks of Copernicus observations of HR1099 in the autumns of 1976 and 1977 and no other flares of a similar or greater magnitude were seen.

The spectra of the radio outbursts along with the proposed star-spot activity on the cooler star indicates that the radio events may result from solar flare-like activity^{1,2,12}. The properties of the 24 September outburst from HR1099 qualitatively resemble that of a large solar outburst scaled up by 2 or 3 orders of magnitude⁵ and this detection of a 2.5–7.5 keV X-ray flare supports this view.

Two other 2–10 keV outbursts have been reported from systems that do not contain a degenerate object (Algol¹³ and the flare star YZ Cmi (ref. 14)); however, this is the first instance of a simultaneous measurement of an X-ray outburst coincident with a radio event. The fact that all these systems are so common indicates that each must represent an important class of X-ray source. Indeed, they may be responsible for some of the short-lived X-ray transient events that are currently being reported (see refs. 15, 16) and refs therein), especially with the possible associations of HR1099 with the transient 4U0336+01 (ref. 17), which was 10 times brighter than the event reported here.

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Discovery of quiescent X-ray emission from HR1099 and RS CVn

OBSERVATIONS with the HEAO 1 satellite have shown that RS CVn binary systems represent a new class of soft X-ray sources¹. While the space density of the normal period RS CVn systems is large⁹, only 26 are known optically¹⁰ and, because of their low X-ray luminosity, only about nine of these should be observable from HEAO 1. We present here our discovery of X-ray emission from HR1099 (ref. 2) and the prototype member of this class, RS CVn itself. We also comment on the

Table 1 Observational properties of RS CVn systems

	Distance (pc)	$V_{\max}$	Period (d)	Spectra type	$F_x(0.2-2.8 \text{ keV})$ (counts $\text{cm}^{-2} \text{ s}^{-1}$)	$L_x(0.2-2.8 \text{ keV})$ (erg s^{-1})
α Aur	14	0.09	104	G5III/G0III	0.07	4×10^{30}
UX Ari	50	6.5	6.4	G5V/K0IV	0.03	2.1×10^{31}
HR1099	33	5.9	2.8	G5V/K0V	0.04	$\sim 1 \times 10^{31}$
RS CVn	145	8.4	4.8	F4III/K0IV	0.01	$\sim 10 \times 10^{31}$

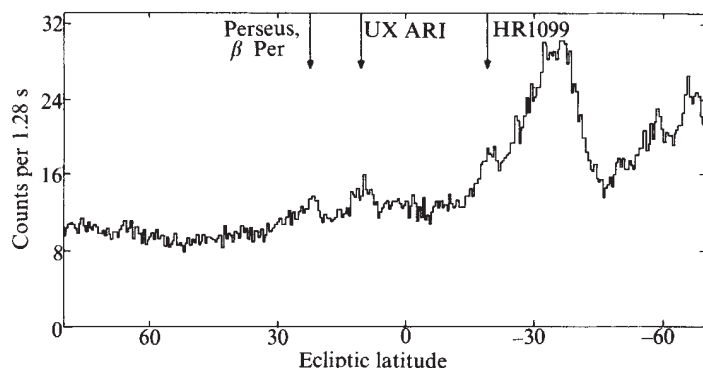


Fig. 1 0.2–1.5 keV data taken on 17 August 1977 superimposed in 0.5° bins. The typical 1σ error is ± 0.5 counts. The feature at -40° is the Eridanus hot spot. UX Ari and β Per are $\sim 1.5^\circ$ off axis; the Perseus cluster is $\sim 3^\circ$ off axis. HR1099 is about 2.5° off axis. The 3° FWHM triangular response of the collimator to HR1099 is distorted by the sharply rising background.

possible nature of the X-ray emission mechanism in these systems.

Our observations were carried out with low energy detectors (LEDs; energy range 0.2–2.8 keV) of the cosmic X-ray experiment³ on board the HEAO 1 satellite. On 17 August 1977, HR1099 was $\sim 2.5^\circ$ from the centre of the scan plane of the X-ray instrumentation (which scans in ecliptic latitude with a period of 30 min). A source was registered in the 3° collimator which was consistent with the location of HR1099 (Fig. 1) and the transient UHURU source 4U0336+01 (ref. 4): the source was not seen on the following day, as expected, as HR1099 was then out of the field of view. Assuming the identification with HR1099, the aspect corrected intensity and corresponding source luminosity are given in Table 1 together with other system parameters.

In addition, HEAO 1 scanned the region of RS CVn in December 1977, and again registered a weak signal in the

LEDs (Fig. 2) consistent with the RS CVn position. In both cases the source was either too weak or too contaminated by a region of enhanced diffuse X-ray background to permit us to construct an X-ray spectrum from our data. However, the total count rates from the detectors are effectively binned into broad spectral bands and both sources are then consistent with the 10^7 K thermal spectrum of UX Ari (ref. 1), and the source luminosities given in Table 1 are computed with this assumed spectrum. The above-mentioned difficulties preclude any more quantitative statement on the source spectra. White *et al.*⁵ have reported the Copernicus observation of a simultaneous radio and X-ray flare from HR1099 thereby confirming our proposed identification of this system.

Walter *et al.*¹ have postulated that the quiescent X-ray emission from RS CVn systems can be explained in terms of coronal emission at a temperature $\sim 10^7$ K. This very hot corona is driven by mass and energy injection from continual flaring associated with the massive starspot complexes in these systems. Such emission has now been observed in the RS CVn systems UX Ari, HR1099, RS CVn and the long period RS CVn system α Aur (Capella)^{6–8}, whose observational properties^{9,10} are summarised in Table 1. As these binary systems exhibit large-scale radio flaring^{11,12}, and optical⁹ and UV flaring¹³, we have predicted¹ that such flaring, in a solar analogy, should be accompanied by X-ray flaring and a corresponding hardening of the X-ray spectrum. The observations by White *et al.*⁵ and the likely relation of the transient 4U0336+01 to HR1099 support this prediction.

We conclude that hot, extensive stellar coronae have now been found in several stellar systems with large starspot¹⁴ complexes. We suggest that other stellar X-ray sources may indicate considerable surface activity and that future X-ray observations could be a means of identifying hitherto unknown RS CVn stars.

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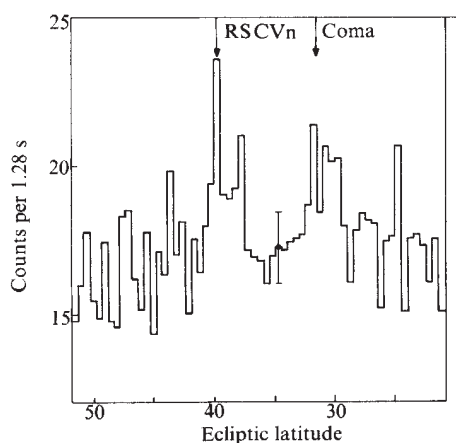


Fig. 2 Superimposition of four scans of RS CVn on 20 December 1977 in 0.5° bins. The energy range is 0.2–1.5 keV. The reduced χ^2 of the fit of the collimator response is 0.8. The Coma cluster (32°) is 2.5° off axis.

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Consequences of narrow cyclotron emission from Hercules X-1

A LINE feature has been observed¹⁻⁴ in the hard X-ray ($E \approx 58$ keV) spectrum of the compact X-ray source Hercules X-1. If this were interpreted as a cyclotron line emission from a strong magnetic field ($B \geq 10^{12}$ G), then the observations could be used to place strict limits on the total source luminosity in the cyclotron line. The salient feature of the observed line emission is that the FWHM is < 12 keV (ref. 1), with a total observed luminosity in the line of approximately 1% of the total X-ray luminosity. The line seems to be pulsed in phase with the 1.24 s hard X-ray pulse². If we understand, even vaguely, the physical conditions at the pole of an accreting neutron star, as in Her X-1, we would expect a lot of cyclotron emission. For a temperature of $kT_e \approx 20$ keV, as inferred from the X-ray spectral data⁵, and a strong magnetic field ($B \approx 5 \times 10^{12}$ G), as inferred by flux conservation, it is easy to excite the electrons, either by collisions or by blue-shifted photo-excitation. For a neutron star with such a magnetic field and a gravitational redshift of 0.1, the fundamental harmonic of the

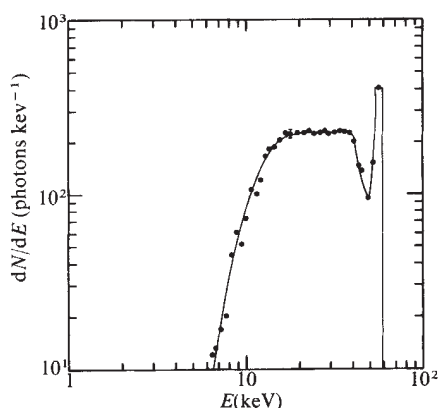


Fig. 1 The spectrum of the radiation that escapes through the shell. The line is drawn through the Monte Carlo points merely to guide the eye. $\tau_{es} = 3$, shell with holes; $E_0 = 56$ keV.

cyclotron radiation appears at approximately 58 keV. In the data of Trümper *et al.*¹, there is some evidence for the second harmonic, tending to give credence to the reality of the interpretation. If the line emission is real and the hard X-ray pulse is due to the occultation of a more isotropic hard X-ray emission by a shell of gas at the neutron star magnetosphere^{6,7}, the calculations presented here indicate that the total source luminosity of the cyclotron line is more than an order of magnitude greater than the observed amount.

McCray and Lamb⁶ and Basko and Sunyaev⁷ have proposed that Her X-1 has a magnetospheric shell of variable opacity in magnetic latitude, to account for the soft X-ray luminosity⁸⁻¹⁰. They predicted⁶ a certain pulse phase relationship between the hard and soft X rays, which was later confirmed by observations¹⁰. McCray and Lamb estimate that the scattering depth of this shell is $\tau_{es} \approx 3-5$. In the cyclotron line source region at the magnetic poles of the neutron star, the electrons are confined to travel nearly parallel to the B -field lines. Thus, a narrow cyclotron line is only emitted at angles that are nearly perpendicular to the field lines. Even if $kT_e = 15$ keV, the line photons must be emitted at angles $\alpha \geq 40^\circ$ to the field lines, so as not to be broader than the observations¹¹. For higher temperatures, the narrow radiation is beamed even more directly at the magnetic equator of the shell.

As the narrowest line is directed toward the thickest part of the shell, it is easy to show that most of the line photons will be

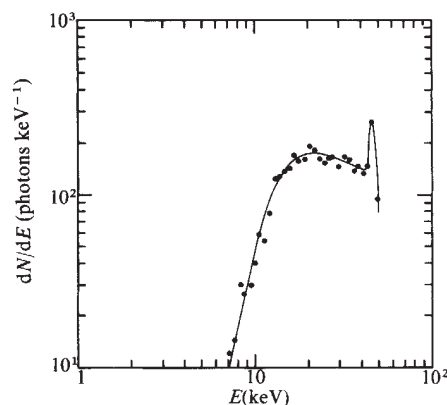


Fig. 2 As in Fig. 1 for the radiation that escapes through the holes in the shell. The holes remove 25% of the total surface area of the shell—see text.

removed from the narrow core of the line by incoherent Compton scattering¹². In fact, averaging over angles at $T_e = 0$ (a good approximation for the conditions in the shell), one finds that the average loss in energy per Compton collision (using the Thomson cross section) is $\langle \Delta E \rangle = -E^2/m_e c^2$, where E is the initial energy of the photon. Superimposed on this steady energy loss is a dispersion of the line distribution because not all photons scatter through the same angles. This produces an important source of broadening¹³, even for a zero temperature gas: $\sigma^2(E) \approx E^2 \cdot \frac{2}{3} [E/m_e c^2]^2$. Thus, the broadening is of the same order as the energy shift. For $\tau_{es} = 4$, an average photon is expected to scatter about 16 times; thus, even a delta function at 58 keV will be scattered into a broad peak centred near 20 keV and having a width of $2\sigma \approx 30$ keV.

So, even if an extremely narrow line can be emitted by the source region, how can such a narrow line survive after scattering through the magnetospheric shell? Within the framework of the shell model, there are two possible ways a photon can leave the system. Either it escapes through the holes in the shell over the magnetic poles where the hard X-ray pulse originates, or it escapes through the shell itself. Photons in the narrow line that escape through the holes must have backscattered at least once since they were originally beamed toward the magnetic equator of the shell. For photons that escape the system through the shell, the only way a narrow line can be maintained is if the broad peak of scattered line photons is sufficiently removed in energy from the position of the original narrow core for the residual peak of unscattered, or primarily forward-scattered photons to be seen above the continuum. I have studied both these escape routes using a Monte Carlo radiation transfer code. The results indicate that a narrow line can escape the system by either route, if the initial energy of the line is $> \sim 20$ keV.

As the simplest possible model of the shell geometry, and following Langer, Ross and McCray¹⁴, I have used a uniformly thick ($\tau_{es} = 3$) and cold spherical shell surrounding a point source of X rays, with symmetrically placed holes over the magnetic poles. The holes remove 25% of the surface area of the shell. The line emission is approximated by injecting monoenergetic photons into the shell at 90° from the centre of the holes. Absorption of the X rays is neglected. Using this model, one may see qualitatively whether narrow features can escape the system; however, quantitative results concerning, for example, the exact source strength of the line, depend critically on the angular distribution of the line emission, which is extremely uncertain because of the unknown geometry and physical conditions in the source region. The main feature we know about the source region is that if a narrow line is emitted, it is emitted perpendicularly to the field lines. This is the only source parameter specified in this model.

The results of this study are shown in Figs 1 and 2. Figure 1 shows the spectrum of the total radiation that escapes through

the shell. In particular, there is a residual line feature which is much stronger than the tail of the scattered radiation at that energy. The initial energy of the line here is $E_0 = 56$ keV. If the total number of emitted line photons were plotted on Fig. 1 using the bin width at the line energy, its photon spectral strength would be 4.8×10^3 photons keV^{-1} . Figure 2 shows the spectrum of the total radiation that escapes through the holes. Again, there is a narrow feature in the spectrum. The energy of this narrow ($\Delta E \approx 3$ keV) feature is exactly the energy of a once backscattered line photon $E' = E_0/(1 + 2E_0/m_e c^2) = 46$ keV. In both cases the total luminosity in the narrow feature is only a small fraction of the total luminosity emitted in the line. Only 5% of the total initial luminosity escapes in the narrow feature through the holes, whereas 11% escapes in the narrow feature through the shell. In any case where there is a scattering medium surrounding the source, the total number of photons that escape the system in the cyclotron line feature is only a small fraction of the total number that were emitted in the line.

A narrow line can escape this idealised model of the Her X-1 geometry. However, if the scattering depth from points on the shell through the 'hole' over the magnetic poles is sufficiently large, photons that would have escaped in the narrow core would Compton scatter again. In this case, one would not see a narrow line core. This sequence may occur, for example, if the opaque accretion column (due to the converging magnetic field lines) over the poles can intercept a large fraction of the photons that would have escaped. Thus, the question of whether a narrow line can, in reality, escape the Her X-1 system, depends on the (presently unknown) exact accretion flow structure near the magnetopause.

These results indicate that if a narrow line can escape the system, then some fraction $> \sim 10\%$ of the available gravitational energy in the accretion is being transformed into the cyclotron line. Note that in this model (even if a narrow line is not seen), a significant amount of the 20–50-keV continuum is merely scattered cyclotron radiation. As the observed line seems to be pulsed in phase with the hard X rays², it seems that the observed emission is cyclotron line radiation that has back-scattered and escaped through the holes in the magnetospheric shell. More detailed models using model-dependent angular emission distributions need to be developed to make better quantitative predictions as to the source luminosity of the cyclotron line.

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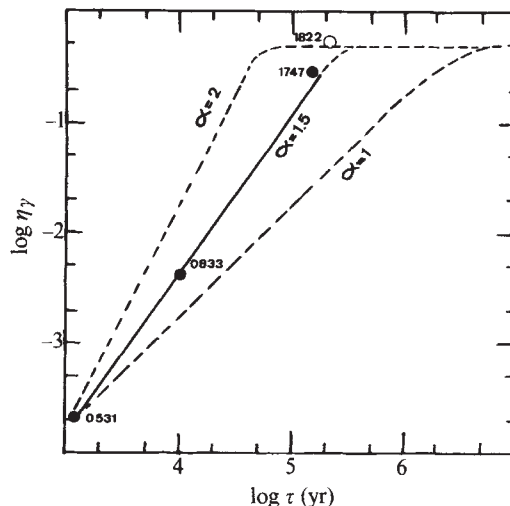


Fig. 1 γ -ray efficiency plotted against apparent age.

fact, because of the poor angular resolution intrinsic to the γ -ray telescopes, the only possibility of identification for individual sources is the time structure of the emission, and the periodical pattern of pulsars is particularly suited. On the other hand, for PSR0531+21 and PSR0833–45, the observed pulsed energy release is essentially in the γ -ray channel, with a ratio between the γ and radio luminosity as high as $\sim 10^6$.

General surveys have been carried out both on SAS 2 and COS B data in an attempt to detect possible γ -ray emission from other known radio pulsars.

The SAS 2 search² was based on a straightforward χ^2 -test on the phase histograms obtained by folding the photon arrival times with the values of the period P and its derivative $\dot{P}$ extrapolated to the epoch of the γ -ray observations: out of 75 objects, PSR1747–46 and PSR1818–04 have been indicated as detectable emitters. A similar analysis has been applied in the COS B pulsar search³ for 32 objects: no positive result was reported and the γ -ray pulsation of PSR1818–04 was not confirmed. However, the discovery and the observation of γ -ray pulsars with this procedure is difficult. Low statistics strongly limits the analysis of the γ -ray data with a chance of obtaining spurious results; moreover, the very precise values of P and $\dot{P}$ necessary were incompatible, in most cases, with the extrapolation over the very long time intervals between the epochs of the radio and γ -ray observations⁴. Part of the difficulties can be overcome if these observations are contemporary or very shortly spaced in time as for PSR1747–46 (ref. 5).

Alternatively, a scanning procedure over convenient intervals of P and $\dot{P}$ can be adopted⁴; a preliminary analysis of the COS B data over six radio pulsars has given a positive result for γ -ray emission from two radio pulsars³: PSR1742–30 and PSR1822–09. We have deduced an approximate value of the γ -ray flux for PSR1822–09 from the published light curve and χ^2 distribution, giving ~ 0.3 that of PSR0531+21.

To gain some insight into the properties of the γ -ray emission from pulsars and to suggest a guide for a rational search of detectable γ -ray pulsars, we use a phenomenological approach.

The known sources have different values for the fraction of the rotational energy which is radiated as γ photons. It is useful to introduce an efficiency parameter η_γ defined as:

$$\eta_\gamma = L_\gamma / \dot{E} = L_\gamma P^3 / (4\pi I \dot{P}) \quad (1)$$

where L_γ is the γ -ray luminosity of the pulsar and $\dot{E}$ the rotational energy loss rate. η_γ may be a function of many parameters (such as period, magnetic field and energy of the emitting particles), and it can be clarified only within the frame of a definite model. At present, however, a consistent theory

Observability of γ -ray pulsars

PULSARS seem to play a major role as γ -ray emitters. Of the 13 objects listed in the first COS B catalogue¹ the two brightest, CG185–5 and CG263–2, have been identified with the Crab (PSR0531+21) and Vela (PSR0833–45) pulsars respectively. This privileged role of pulsars as identified γ -ray sources could be simply related to observational reasons: in

explaining the complex phenomenology of pulsar activity is not available, the theoretical investigations having been dependent on the radio emission data only. The weak dependence of the period of the γ -ray luminosity observed for PSR0531+21 and PSR0833-45, seems to favour the general framework initially proposed by Sturrock⁶ and developed by Ruderman and Sutherland⁷. They suggest that positrons are accelerated up to very high energy ($\approx 10^{12}$ eV) across a potential drop in an electrostatic gap developing on the polar caps, close to the neutron star surface; there, they move along the magnetic field lines and emit γ -rays by curvature radiation mechanism. The problem of emission and transfer of the γ -radiation in the pulsar environment has been recently treated by Salvati and Massaro⁸, who take into account the star rotation and, by considering a dipole field geometry, can produce a solution which is accurate to the first order in the rotational frequency and includes the effects due to aberration, Doppler shift and pair-production absorption. A comparison of their numerical results carried out for the specific case of PSR0833-45 shows a good qualitative agreement with the COS B data.

According to Ruderman and Sutherland⁷, the total power in high energy positrons which can be provided by the gap acceleration, depends on the pulsar parameters as

$$W \propto B^{6/7} P^{-15/7} \rho^{4/7} \quad (2)$$

where B is the magnetic field strength at the neutron star surface and ρ the curvature radius of the magnetic field lines in the same region. When the initial energy of the emitting particles is sufficiently high, a relevant fraction of this power (of the order of 0.5) can be radiated as γ -rays and can be considered to have very little dependence on P . From equations (1) and (2), we deduce for the γ -ray efficiency:

$$\eta_\gamma \propto \rho^{4/7} P^{6/7} \dot{P}^{-1} \quad (3)$$

While Ruderman and Sutherland⁷ suggested ρ values of the order of the radius of the neutron star, Salvati and Massaro⁸ assumed ρ was the curvature radius of the last open field line. In the first case ρ is not a function of P ; in the second, a dependence $\rho \propto P^{1/2}$ is implied. The relation (3) becomes, respectively,

$$\eta_\gamma \propto P^{6/7} \dot{P}^{-1} \quad (4a)$$

or

$$\eta_\gamma \propto P^{8/7} \dot{P}^{-1} \quad (4b)$$

As the difference is not significant at this stage, we take

$$\eta_\gamma \propto P/\dot{P} \propto \tau \quad (5)$$

where τ is the apparent age of the pulsar. It follows that old

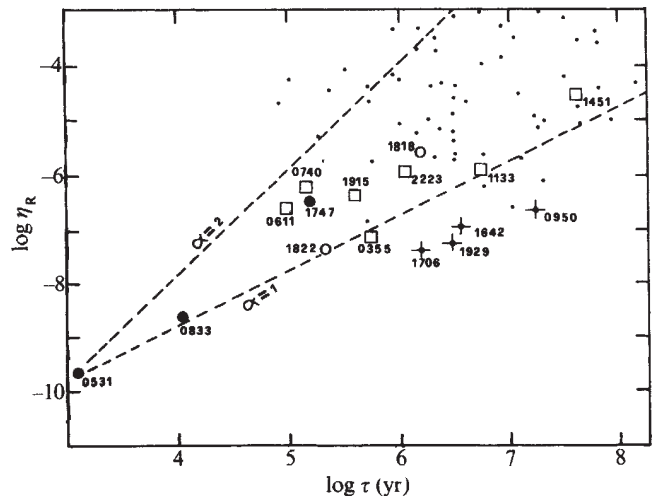


Fig. 2 Radio efficiency plotted against apparent age. $\nu = 400$ MHz; $\Delta\nu = 400$ MHz. ●, The normalising points; ●, sources with $\tau > 10^6$ yr and $\phi_\gamma/\phi_{0531} > 0.5$; □, best candidate sources.

pulsars should be more efficient than young ones in converting rotational energy to γ rays.

Figure 1 shows η_γ plotted against τ for the observed γ -ray pulsars (the moment of inertia of the neutron star has been assumed to be identical for all the objects and equal to 10^{45} g cm²). It can be seen that the general trend of increasing efficiency with apparent age is confirmed. The experimental points for $\tau \leq 2 \times 10^5$ yr are well fitted by a power law $\eta_\gamma \propto \tau^\alpha$ with $\alpha \approx 1.5$, suggesting an even steeper law than equation (5). This behaviour of η_γ is basically supported by the Crab and Vela data together with that of PSR1747-46; the point corresponding to PSR1822-09 has been deduced adopting the flux value $\phi_\gamma = 0.3\phi_{0531}$. We have imposed an asymptotic value of $\eta_\gamma = 0.5$ for τ greater than 2×10^5 yr; nothing can be said *a priori* about the behaviour of η_γ in this range and the possibility of a decreasing efficiency for the γ -ray emission or even a switch-off at large τ values cannot be excluded.

Figure 2 shows a similar plot to Fig. 1 for the radio efficiency η_R ($\nu = 400$ MHz, $\Delta\nu = 400$ MHz), built using the data reported in the Taylor and Manchester tabulation⁹ updated to February 1977. The data also suggest in this case, although with a rather high spread, a trend of increasing η_R going from young to old pulsars.

By using η_γ as function of τ as given by Fig. 1 and the values of τ and the distance D as listed in the Taylor and Manchester tabulation, we can estimate ϕ_γ for 88 pulsar by the equation:

$$\phi_\gamma/\phi_{0531} = \eta_\gamma (\dot{E}/D^2)(D^2/L_\gamma)_{0531} \quad (6)$$

The assumption of an identical duty-cycle and energy spectrum for all the pulsars has been made.

Figure 3 shows the pulsars for which the expected value of ϕ_γ/ϕ_{0531} is greater than 10^{-3} . It is difficult to set a threshold value of ϕ_γ for the detectability by the COS B satellite because of the dependence on the location of the source in relation to the level of the continuous background. As a first approximation, this threshold can be set at 0.1 of the PSR0531+21 γ -ray flux; but, because of the indetermination of pulsars' distance (up to a factor 3) when derived from the dispersion measure, the predicted luminosity could be in error by a factor as high as 10.

With this in mind, Fig. 3 indicates the following: three sources constitute the normalising points. The PSR1818-04 expected luminosity seems to be about a factor of 30 lower than that claimed by the SAS 2 observation; we believe, however, that, because of the procedure followed in the analysis, the effect observed by the SAS 2 group could be explained by a chance fluctuation of the background.

Table I Estimated and observed γ -ray fluxes for 15 radio pulsars

PSR	P (s)	τ (yr)	ϕ_γ/ϕ_{0531}	
			Est.	Obs.
0833-45	0.089236	1.13×10^4	3.3	3.3
0531+21	0.033098	1.24×10^3	1.0	1.0
0950+08	0.253065	1.73×10^7	≤ 1.0	
1929+10	0.226517	3.10×10^6	≤ 0.8	
1642-03	0.387689	3.45×10^6	≤ 0.8	
1706-16	0.653050	1.62×10^6	≤ 0.7	
0355+54	0.156380	5.64×10^5	0.4	
1747-46	0.742352	1.68×10^5	0.3	0.3
0740-28	0.166752	1.57×10^5	0.3	
1822-09	0.768950	2.35×10^5	0.2	~ 0.3
0611+22	0.334919	8.88×10^4	0.15*	
1915+13	0.194626	4.28×10^5	0.06	
1133+16	1.187911	5.04×10^6	0.05	
1451-68	0.263377	4.35×10^7	0.05	
2223+65	0.682533	1.14×10^6	0.05	

* $D = 1,500$ pc.

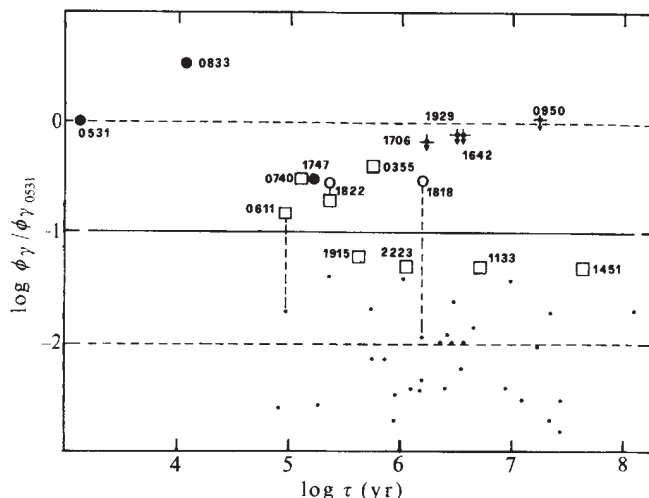


Fig. 3 Predicted γ -ray fluxes from radio pulsars. Symbols as in Fig. 2.

For PSR1822–09 the expected and the estimated values for the flux agree reasonably well within the limits of the indetermination of our analysis.

The four sources at $\tau > 10^6$ yr and with predicted $\phi_\gamma/\phi_{\gamma 0531} > 0.5$ (PSR1706–16, PSR1929+10, PSR1642–03, and PSR0950+08), indicated in Figs. 2 and 3, all show abnormally low efficiency η_R at radio frequency (Fig. 2); this could be due to an underestimation in the distance values deduced from the dispersion measure, and given as between 100 and 170 pc. If so, the predicted $\phi_\gamma/\phi_{\gamma 0531}$ values could be overestimated and in reality they should be nearer the visibility limit or even below it.

Two other pulsars seem to be good candidates for detection: PSR0740–28 and PSR0355+54. Of the 18 sources with $0.01 < \phi_\gamma/\phi_{\gamma 0531} < 0.1$, because of the indetermination on the assumed distance a fraction about equal to 1/3 (that is five or six sources), could be at the limit of detectability or above it. One of them, PSR0611+22, if associated with the SNR IC443, would have a distance of $\sim 1,500$ pc as deduced from optical measurements instead of the 3,500 pc given by DM measurements; this would imply a $\phi_\gamma/\phi_{\gamma 0531} > 0.1$ instead of 0.02 as shown in Fig. 3.

Table 1 lists the radio pulsars for which the expected γ -ray flux is higher than the 5×10^{-2} of PSR0531+21; these are the most promising candidates for observations by COS B satellite. Naturally, a positive identification strongly depends on the availability of accurate inputs from radio data.

For very young pulsars, such as Crab and Vela, the γ -ray efficiencies are low, whereas η_γ seems to increase rapidly with the apparent age up to a value corresponding to a dominant role of γ -ray emission in pulsar energy release. It is essential to verify this trend and to investigate if a strong γ -ray emission is still persisting for old pulsars ($\tau \approx 10^6$ yr).

As for the characteristics of the γ -ray emission from pulsars, they seem to be better explained by a general framework of models where the emitting particles are accelerated up to very high energies very close to the surface of the neutron star, and the curvature radiation is the process responsible for the γ -ray flux⁸. More detailed models should also be able to explain how the behaviour of η_γ varies with τ .

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Continuous measurements of nitrous oxide in the troposphere

CONTINUOUS measurements of N_2O at the ground surface by Brice, *et al.*¹ have been interpreted as “evidence for the existence of an appreciable, but unidentified, ground surface sink mechanism”. They based this conclusion on an apparent regular diurnal variation in the concentration of N_2O in the air 5 m above the ground, observed during the periods 24–29 July and 4–8 August 1976. “A minimum was nearly always recorded in the early hours of the morning and a maximum was recorded in the late afternoon or evening.”¹ In contrast to these results, Cicerone, *et al.*² reported N_2O measurements made during 25–27 May and 29 August–1 September 1977 which show exactly the opposite behaviour, suggesting instead “that the 1200–1800 h time period sees significantly (at the 1σ level) lower N_2O concentrations than does the period 0000–0600 h LT”. Contrary to both these results, we made continuous measurements of tropospheric N_2O for a full year and found no significant diurnal variation in the atmospheric level of N_2O . The only diurnal variation we observed was an experimental artefact created by the diurnal variation in the ambient temperature affecting the sensitivity of the instrument.

Our measurements were made at a fixed, ground-level site during the period from 6 June 1976 to 3 June 1977, using an electron capture gas chromatograph similar to those used by

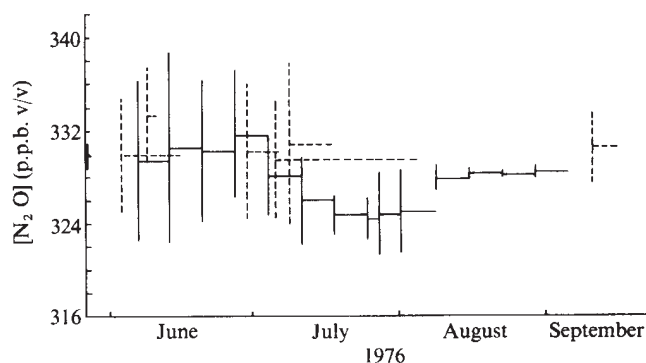


Fig. 1 Nitrous oxide concentrations for the summer of 1976. Solid line: weekly average N_2O concentrations from continuous monitoring station. Dashed lines, average N_2O concentrations from discrete samples collected over North America and the Pacific Ocean.

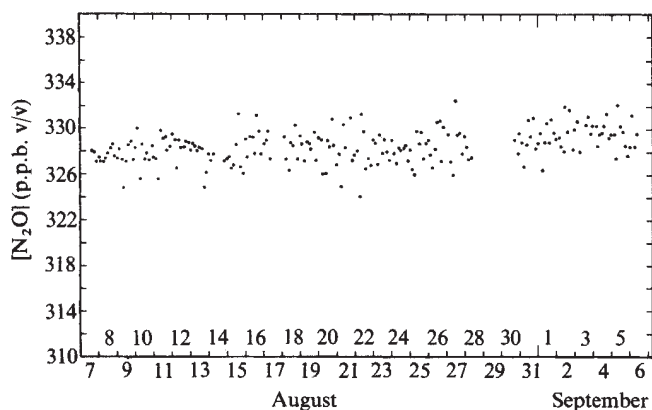


Fig. 2 Nitrous oxide concentrations (3-h averages) for the period 7 August–6 September 1976.

Brice and Cicerone. Our analytical methods for measuring N_2O have been detailed elsewhere³. We consider here the results of the first three months of continuous measurements, from 6 June to 6 September, 1976. The results from the summer of 1976 show a very stable tropospheric N_2O level (Fig. 1), with the concentration varying little from the value of 330 p.p.b. v/v (p.p.b. is parts per 10^9). These measurements show little variation in the tropospheric concentration of N_2O at our continuous monitoring site in eastern Washington, and they also demonstrate that the troposphere over North America and the Pacific Ocean was well mixed with regard to N_2O during this period. In fact, the tropospheric N_2O measurements which we have made over the past 3 yr (ref. 4) show the same homogeneous distribution from the Arctic Ocean to the South Pole.

The variation in our continuous N_2O measurements (Fig. 1, vertical error bars represent 1σ) was greatest in June, only half as great in July and early August, and was almost nonexistent between 7 August and 6 September 1976. This was not due to real atmospheric conditions, but to improvements in our analytical methods as the summer proceeded.

The measurement station was originally set up in an unheated trailer in the country, with standards run twice a day, in the morning and the afternoon, similar to the procedure of Brice, *et al.*¹. However, we soon observed that our measurements showed a marked diurnal variation not only for N_2O , but also for CF_2Cl_2 (F-12) and $CFCl_3$ (F-11), with the maxima occurring in the afternoon and the minima in the early morning. If the variation had occurred for N_2O alone we might have concluded that it was a real phenomenon. However, it is extremely unlikely that such unreactive and non-biological compounds as F-11 and F-12 would show a marked diurnal variation, so we immediately determined that the apparent diurnal cycle was an artefact of our experimental methods.

Because we believed the large temperature variations in the unheated trailer were probably responsible, we ran analyses on a standard cylinder continuously for two days. As we expected, the sensitivity of the gas chromatograph (as shown by the peak heights of N_2O , F-11, and F-12) showed a direct correlation with the ambient temperature inside the trailer. To correct this we installed a thermostatically-controlled heater in the trailer at the beginning of July. The result can be seen in Fig. 1, as the variation in our N_2O measurements (and in our F-11 and F-12 measurements) was immediately halved. However, we were still unable to completely eliminate the problem caused because the standards were being run during the daytime, when the temperature inside the trailer was at a maximum. Therefore, samples of ambient air run during the night were analysed at a lower (trailer) temperature than the standards, so they seemed to have lower concentrations of N_2O , F-11, and F-12 than the samples run in the daytime, which were analysed at

the same (trailer) temperature as the standards. To solve this problem we developed an automated system to run samples from the standard cylinder every 3 h. This system was put into operation 7 August 1976, and the results were dramatic.

The variation in the atmospheric concentration of N_2O virtually disappeared (Figs 1 and 2). The concentration for the month of 7 August–6 September 1976, based upon all 207 3-h averages, was 328.5 ± 1.5 p.p.b. v/v N_2O (Table 1). This variation is scarcely larger than the precision limits of the measurements, which were ± 1.0 p.p.b. during the period in question.

The small variation found over this 31-day period agrees with the results of our other N_2O measurements⁴. This homogeneity in the tropospheric mixing ratio of N_2O indicates a relatively long atmospheric lifetime for the compound. Using Junge's empirical formula for relating atmospheric variability to atmospheric lifetime⁵, the results of the 7 August–6 September 1976 measurements indicate an atmospheric lifetime of at least 32 yr for N_2O . This agrees well with the results of R. F. Weiss (personal communication) who found a relative variation of less than 0.5% for tropospheric N_2O , implying a residence time >28 yr.

Our results for this period also showed no significant diurnal variation for N_2O (Fig. 3). As the sample and reference analysis times were the same every day, we averaged the measurements within each 3-h period over the entire 31 days. There is no significant difference between the N_2O concentrations found during the daylight and dark hours. The average N_2O concentration for the hours 0745–1945 was 328.9 p.p.b. v/v, and the concentration for the period 2245–0445 was 328.7 p.p.b. v/v N_2O . The periods 0445–0745 and 1945–2245 represent the times of sunrise and sunset, respectively, and they also show the lowest N_2O concentrations of any periods of the day. This is probably not a real atmospheric phenomenon, but the result of the rapid heating and cooling of the trailer when the Sun rises and sets. Therefore, even with the attempts to stabilise the temperature inside the trailer, and running standards automatically every 3 h, we were not able to completely eliminate the temperature effects on the sensitivity of the gas chromatograph.

Nonetheless, our results establish fairly conclusively that there was no significant diurnal variation in the tropospheric concentration of N_2O over northwestern USA during this period. This apparently disagrees with the results of Brice *et al.*¹ who found a daytime maximum during the same period while making measurements in England, and Cicerone, *et al.*² who saw a maximum at night during two periods of measurement in Michigan during the spring and summer of 1977.

The variations found by Cicerone *et al.*² are quite small, and could simply be the result of the limited number of samples

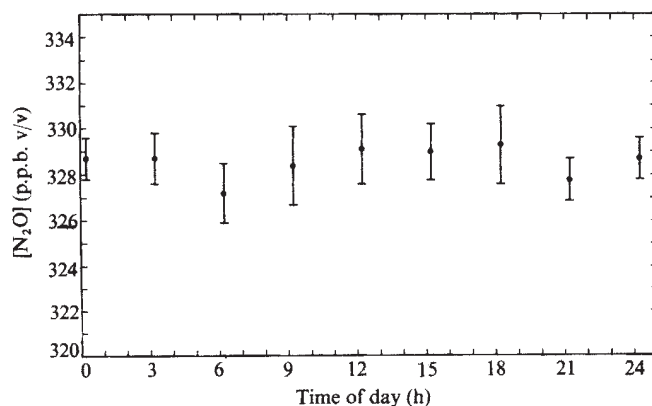


Fig. 3 Average diurnal variation in the concentration of N_2O during the period 7 August–6 September, 1976.

Table 1 Nitrous oxide concentration

	$\bar{x}$	s	n
All 3-h averages 7 August–6 September 1976	328.5 ± 1.5		207
Relative standard deviation (in a 3-h period)	<0.3%		207
Relative standard error (in a 3-h period)	0.1%		207

Concentrations in p.p.b. ($\times 10^{-9}$) v/v.

involved in their study of diurnal variation. However, the variations found by Brice *et al.*¹ are large, usually exceeding 10% between the daytime maximum and the night-time minimum, and are the result of a much larger sample than Cicerone used. From their data it seems that at least part of the variation they observed is not real, but simply due to the same sort of temperature-sensitivity variations which gave us such difficulties. As in our early measurements, they also ran standards in the morning and afternoon (at 0900 and 1500), when the temperature around the instrument was probably higher than it was at night.

We do not rule out the possibility that the diurnal cycle found by Brice *et al.*¹ may be partly due to real atmospheric variations. Recent experimental results by Blackmer and Bremner⁶ indicate "that soils have a significant capacity for uptake of N_2O , and may represent an important natural sink for atmospheric N_2O ". Furthermore, Cicerone *et al.*² found direct experimental evidence that soils from the same plot of ground can both consume and emit N_2O depending upon factors such as their moisture content and the intensity of incident radiation. Therefore, the potential exists for significant sources and sinks of N_2O in soils, depending on properties such as pH, moisture content, nitrate levels, temperature, and the level of readily decomposable organic matter. Because all these factors are independently variable in real soils, the capacity of soils to consume or emit N_2O will also be highly variable, so it is impossible to make unconditional statements about the role of soils in the global N_2O cycle. However, we do not think that it is likely that they could be responsible for a variation in the N_2O concentration as large as 10–20% at a distance of 5 m above the ground.

Our results do not prove that soils do not act as a source or sink for N_2O , nor that the tropospheric concentration of N_2O does not show a diurnal variation in some conditions. However, they make it clear that no such variations occurred over northwestern USA during the summer of 1976. The measurements also indicate that without any local sources or sinks for N_2O (a condition of Junge's model⁵ for estimating atmospheric lifetimes) the atmospheric lifetime of the compound is 32 yr or greater. This relatively long lifetime for N_2O is important, because it indicates that any perturbations to the natural N_2O cycle will take longer to be felt, but will be of correspondingly greater impact and duration once they take place. Therefore, the effects of increased anthropogenic nitrogen fixation might not be apparent for 50 or 100 yr, but once they develop they will remain with us for a long time, regardless of any remedies we may then attempt.

Consequently, we must continue to study the cycle of N_2O in the environment to determine the possible extent of the danger before we are once again made aware of the fragility of natural environmental cycles to anthropogenic incursions.

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Indian summer monsoon cyclogenesis and its variability

MONSOON disturbances forming over the north Bay of Bengal and moving towards the WNW across northern India are the main rain producers of the Indian summer monsoon. There are some (monsoon) seasons when these disturbances fail to develop in adequate number and some that do form move towards the northwest or north—these are periods of drought. Understanding how monsoon disturbances develop and why they sometimes do not is an interesting scientific and an important economic problem. Studies so far on monsoon cyclogenesis^{1,2} have shown that the monsoon atmosphere is not baroclinically unstable. Shukla¹ obtained barotropically unstable upper tropospheric modes and emphasised the role of the conditional instability of the second kind (CISK) but failed

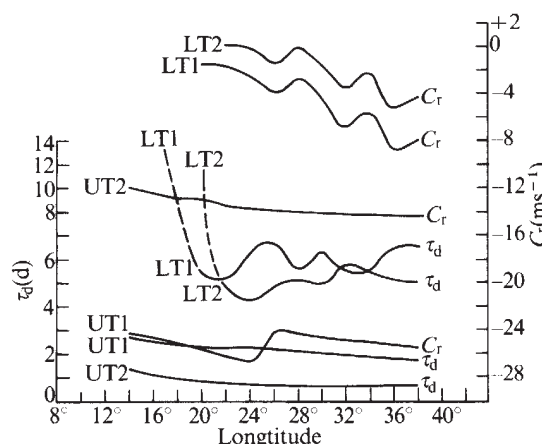


Fig. 1 Growing modes in the lower and upper tropospheres: LT 1,2, lower tropospheric modes; UT 1,2, upper tropospheric modes. τ_d , Doubling time; C_r , phase speed.

to get a preferred scale of maximum growth for lower tropospheric disturbances. R.N.K. *et al.*² found slowly growing barotropically unstable modes in the lower troposphere corresponding to monsoon disturbances. Mak³ studied the baroclinic instability of monsoon zonal flow with a superposed meridional component, but the meridional winds required for instability were large compared to observed winds. Lorenz⁴ studied the barotropic instability of Rossby waves and found that shorter waves can be barotropically unstable. V.S. *et al.*⁵ showed that the superimposition of a long stationary wave, of the scale of wave number two, on monsoon zonal flow yielded fast growing upper tropospheric modes and lower tropospheric modes of scale 2,500 km, a doubling time of 5 days and slow westwards movement. These correspond well to the observed monsoon disturbances. We report here a combined barotropic–

baroclinic stability analysis of monsoon zonal flow with a superimposed long stationary wave (global monsoon) using a two-level quasi-geostrophic model. We show that the phase of the global monsoon wave is a factor in deciding whether a particular year is good for monsoons or for drought.

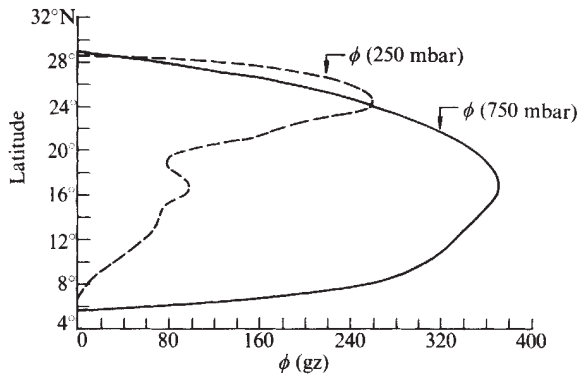


Fig. 2 Amplitude profile of a lower tropospheric growing mode.

The perturbation potential vorticity equations at levels 1 and 3 are

$$\begin{aligned} & \left(\frac{\partial}{\partial t} + U_1 \frac{\partial}{\partial x} + \bar{v}_1^* \frac{\partial}{\partial y} \right) \left\{ \zeta'_1 + \frac{S_2}{f_0} (\phi'_3 - \phi'_1) \right\} \\ & + u'_1 \left\{ \frac{\partial \bar{\zeta}_1^*}{\partial x} + S_2 (\bar{v}_3^* - \bar{v}_1^*) \right\} \\ & + v'_1 \left\{ \beta - \frac{\partial^2 v_1}{\partial y^2} + \frac{\partial \bar{\zeta}_1^*}{\partial y} - S_2 (v_3 - v_1) \right\} \\ & = - \frac{S_2}{f_0} \frac{R}{c_p} \frac{\Delta p}{p} \dot{Q}'_2 \end{aligned}$$

and

$$\begin{aligned} & \left(\frac{\partial}{\partial t} + v_3 \frac{\partial}{\partial x} + \bar{v}_3^* \frac{\partial}{\partial y} \right) \left\{ \zeta'_3 - \frac{S_2}{f_0} (\phi'_3 - \phi'_1) \right\} \\ & + v'_3 \left\{ \frac{\partial \bar{\zeta}_3^*}{\partial x} - S_2 (\bar{v}_3^* - \bar{v}_1^*) \right\} \\ & + v'_3 \left\{ \beta - \frac{\partial^2 v_3}{\partial y^2} + \frac{\partial \bar{\zeta}_3^*}{\partial y} + S_2 (v_3 - v_1) \right\} - \frac{f_0 \omega'_4}{\Delta p} \\ & = \frac{S_2}{f_0} \frac{R}{c_p} \frac{\Delta p}{p} \dot{Q}'_2 \end{aligned}$$

where $v_1(y)$, $v_3(y)$ are basic zonal winds at levels 1 (250 mbar) and 3 (750 mbar), $\bar{v}_{1,3}^*$ are the local meridional components associated with a stationary wave, ϕ' is the perturbation geopotential and u' , v' are the perturbation wind components,

$$\begin{aligned} \zeta' &= \frac{1}{f_0} \nabla^2 \phi', & S_2 &= \frac{f_0^2}{\Delta p^2 \sigma_2}, \\ \sigma &= - \frac{\alpha}{\theta} \frac{d\theta}{dp}, & f &= f_0 + \beta y \end{aligned}$$

and $\dot{Q}'_2$ is the perturbation heating.

We assume wave solutions of the type $\phi' \sim \phi(y) \exp \{i(kx - kct)\}$ where $\phi(y)$ and c may be complex and look for the conditions in which c becomes complex. We use a β -plane centred at 17°N and a channel enclosed between 5 and 29°N. The basic assumption in this simplified approach is that the wavelength of the perturbation is small compared to the wavelength of the stationary wave, so that the meridional component (of the superimposed stationary wave) only slowly

varies within a scale length of the perturbation. We treat it as constant. We thus specify the phase of the stationary wave over Indian longitudes and thereby include only the corresponding meridional component in our stability analysis. For continuity, we assume that this meridional component in the upper troposphere (250 mbar) is of the same magnitude as the meridional wind in the lower troposphere (750 mbar) but is of opposite sign. These meridional components as well as the perturbation geopotential vanish at the two latitudinal boundaries. The vertical p -velocity vanishes at the top bounds and equals the frictional vertical motion at the bottom boundary. After substituting the wave solutions, we now write equations (1) and (2) in a finite difference form at different latitudes using a one degree grid. Thus, we get $2n$ (n being the number of latitudes) coupled equations in $2n$ unknowns. The problem of solving these $2n$ simultaneous equations can be written in matrix notation⁶ as

$$(P - cQ)(\Phi) = 0$$

or

$$(PQ^{-1} - cI)(Q\Phi) = 0$$

where P and Q are the matrices formed with the coefficients of Φ . The problem then reduces to finding the eigenvalues of the matrix PQ^{-1} , which we have done numerically^{6,7}.

A stability analysis has been carried out using a realistic zonal wind profile $U(y, p)$ and a stationary wave of wavelength 180° long; its phase is such that the maximum southerlies in the lower troposphere and maximum northerlies in the upper troposphere occur over Indian longitudes. Amplitudes of 5 m s⁻¹ and 7 m s⁻¹ were used. The analysis yielded fast-growing upper tropospheric easterly waves and lower tropospheric monsoon disturbances of scale around 2,500 km, a doubling time of 4–6 days (Figs 1 and 2), and a slow westwards phase speed lying between -1 and -4 m s⁻¹.

The heating contrast of the Asian Continent with respect to the Pacific Ocean results in an east-west circulation and a consequent global monsoon of wave number two scale (ref. 8). R.N.K.^{9,10} postulated that a colder than normal Asian Continent and a warmer than normal Pacific Ocean can lead to a weakening or eastwards shift of this east-west circulation and that such a thing happened during the drought of 1972. Alexander *et al.*¹⁰ showed some relation between the equatorial pressure gradient across the Pacific in the pre-monsoon months and the monsoon rainfall. The east-west circulation shifted eastwards¹¹ during the drought of 1972. During some abnormal summers the east-west pressure gradient across the equatorial Pacific is weaker than normal, leading to weaker than normal equatorial easterlies, less upwelling, higher ocean temperatures, increased convection and cyclogenesis, warming of the atmosphere and an eastwards shift of the rising limb of the east-west circulation. Charney *et al.*¹² have postulated that much of the inter-annual variability of the monsoons may be due to changes in the bottom boundary conditions, such as sea surface temperature. In view of these arguments, we consider the effect of such an eastwards shift of the stationary wave on

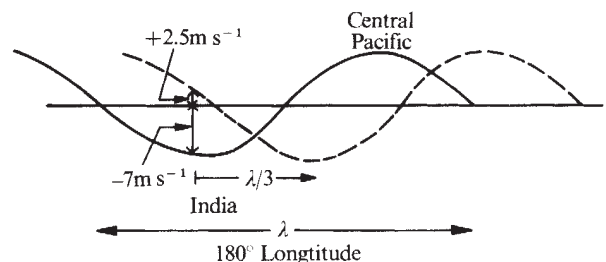


Fig. 3 Schematic diagram showing the stationary monsoon wave in the upper troposphere during normal monsoon (solid line) and the phase-shifted stationary wave during drought (dashed line).

monsoon cyclogenesis over India. When the stationary wave with an amplitude of 7 m s^{-1} shifts eastwards by 60° long (one-third of the wavelength) a meridional component of about $+2.5 \text{ m s}^{-1}$ (southerly) in the upper troposphere and of -2.5 m s^{-1} (northerly) in the lower troposphere (Fig. 3) is obtained. In fact, observations during breaks in the monsoon indicate southerly components in the upper troposphere and some northerly flow in the lower levels. We have repeated the combined barotropic-baroclinic stability analysis with such a phase shifted (by 60° long) superimposed stationary wave and we get no growth of disturbances in the lower troposphere, showing that such a phase shift has an inhibiting influence on monsoon cyclogenesis. Even in this case, however, we obtained some unstable upper tropospheric modes but with reduced growth rates. Through this analysis we have demonstrated one of the important interactions of the global monsoon and the regional monsoon.

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Oil-shale kerogen: low temperature degradation in molten salts

GREEN RIVER oil shale has been found to be converted to soluble products by the action of a sodium tetrachloroaluminate melt¹. The function of the tetrachloroaluminate melt has been shown experimentally to include chemical effects in addition to its role as a thermal transfer agent. The results described here when compared to a conventional retorting process or a shift reaction², are distinctly different, implying that the reaction could be thermally dependent, but results indicate the process involved is not a pyrolysis. Model studies have demonstrated that aromatic moieties are relatively inert to the solvent system described; however, aliphatic materials containing carbon in other than sp^3 hybridisation are especially reactive. The mechanism of degradation seems to involve a mode of intramolecular disproportion catalysed by the tetrachloroaluminate melt's ability to stabilise the resulting short-lived intermediates until the macromolecules involved have been sufficiently reduced in size to become soluble in conventional solvents.

This work is concerned with the degradation of Green River oil shale, principally at 320°C , using a sodium chloride saturated tetrachloroaluminate melt. To examine this degradation the

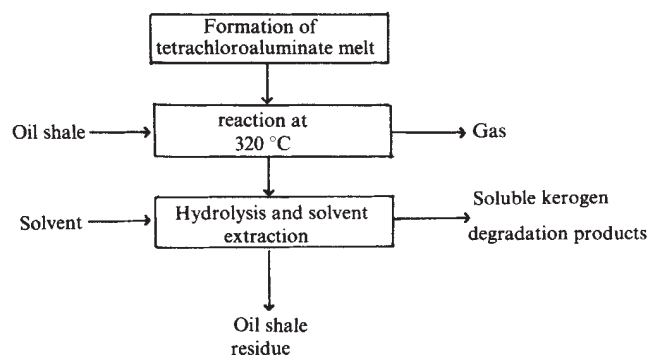
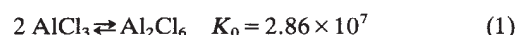


Fig. 1 Flow diagram of reaction process.

acid-base properties of the solvent, in this case the tetrachloroaluminate melt, must be fully understood. The three principal equilibria linking the major species present are²



where K_0 , K_2 and K_M are the mole fraction equilibrium constants at 175°C . The dominant acid-base equilibrium is described by equation (3) with Cl^- and Al_2Cl_7^- the Lewis base and acid respectively.

A simplified flow diagram of the reaction process is shown in Fig. 1. Green River oil shale having a Fischer assay of 66 gal ton^{-1} gave 58% conversion of the organic carbon present. In comparison dry heating of 52.6 and 65 gal ton^{-1} assay shale at 300°C for 30 min resulted in, respectively, only a 2.4% (ref. 3) and 3.3% (ref. 4) conversion. The conversion ratio of Green River shale (assay 66 gal ton^{-1}) processed by the shift reaction developed by Cummins and Robinson⁵ at 325°C for 30 min similarly resulted in only a 14% conversion of the organic carbon originally present. Some of the parameters which had to be considered in determining the specific activity of a reaction were the residence time, temperature, the amount of sample which could be introduced into a constant volume of melt, and the overall effect of particle size upon the amount of solubilised kerogen. The solvent system was maintained at a constant 71.6 g , or 0.375 mol of sodium tetrachloroaluminate throughout each of the sequences used to determine the various parameters. The effect of residence time upon the total yield of solubilised organic materials from Green River kerogen by the melt are shown in Fig. 2a. An optimum time for the sample to remain in contact with the molten salt seems to be 25 min. Although the yield at this point was lower, the increased yield at longer residence times did not compensate for the longer times needed to complete each reaction.

Temperature effects on both the untreated Green River shale and a carbonate-free concentrate⁶ are compared in Fig. 2b. No difference was observed between the two throughout the entire region, with 320°C giving the highest yields of solubilised kerogen.

The effect of the amount of sample treated by a given quantity of molten salt was investigated, and the results are shown in Fig. 2c and d. In Fig. 2c untreated Green River shale was used, while in Fig. 2d carbonate-free Green River shale was used. Whether carbonates are present in the shale does not seem to be very important in the overall reaction efficacy⁷. The reactions were typically run using Green River shale ground to 200 mesh (no study has been performed on larger mesh shale).

When the analytical results were examined from each product it was discovered that a significant amount of dehydrogenation had occurred in the products as compared with the original shale. In addition, a small amount of chloride was shown by

analysis to have been incorporated into the benzene and methylene chloride soluble products. It seemed possible to introduce hydrogen into the products as they were being formed *in situ*. Preliminary results indicated that it should be possible to introduce this hydrogen product up to a higher level than that of the original shale. Analysis of the *in situ* soluble kerogen products was at first disturbing. Hydrogen was indeed being taken up by the products; however, the overall carbon-hydrogen ratio of these products never exceeded that which was present in the original shale. Assuming that the postulated mechanism is functioning this result can be explained: hydrogen is being lost during the reaction as hydrochloric acid, while kerogen radicals are forming in the solvent system. The production of these radicals is a function of the molten salt's ability to produce these radicals and at no time can the introduction of new hydrogen, presumably through a radical mechanism, exceed the kerogen radical population. Hence slightly less than the original hydrogen can be introduced in the best conditions.

A carbon balance was obtained for both the untreated shale and carbonate-free concentrate. A carbon balance provided information as to the whereabouts of the original kerogen organic carbon and provides a means to determine the amount of kerogen which has been removed. Not all the organic carbon originally present was recovered, and presumably the remainder was either in the aqueous hydrolysis layer or was lost in the gas evolved.

To clarify the mechanism by which Green River kerogen is solubilised, several different molten salt systems were investigated. The carbonate-free concentrate⁸ was processed in a molten salt melt composed of three alkali acetates⁹. The condition of the reactions were otherwise identical with that of the tetrachloroaluminate melt. Alkali acetate molten salts have been found to possess a low level of chemical activity while being able to function as excellent thermal transfer agents. Little solubilised kerogen was recovered from the reaction. The results of this process indicate that the tetrachloroaluminate melt does not function primarily as a thermal agent.

To observe a totally Lewis acid catalysed chemical degradation, any thermal activity had to be minimised. A eutectic of ethylpyridinium bromide and aluminium chloride¹⁰, a liquid at ambient temperature, was used so that minimal thermal activity

towards the kerogen was present. Ethylpyridinium halide-aluminium halide molten salts have been shown¹¹ to possess high levels of chemical activity at low temperature owing mainly to the relatively high 'acidity' established by 2:1 molar ratios of aluminium halide to pyridinium salt. When a sample of Green River shale was processed, no evidence of any increased solubilisation of the kerogen was observed.

In conclusion, the tetrachloroaluminate melt, while being an excellent thermal medium, does not function simply as a means of thermal degradation. On the other hand, any chemical activity present in an ethylpyridinium bromide aluminium chloride melt at ambient temperature is insufficient to effect degradation of the kerogen.

Recent model studies have uncovered some of the characteristics and effects on organic compounds of tetrachloroaluminate melts at 300 °C. We have found that aromatic systems are relatively inert in sodium chloride saturated tetrachloroaluminate melts, and aliphatic compounds such as decalin do not undergo any appreciable interaction. When cholesterol, which is quite similar in structure to a number of compounds isolated from bitumen, in particular the triterpenoids isolated by Robinson and others¹², was placed into a tetrachloroaluminate melt, an immediate reaction occurred. Preliminary results show that side-chain removal and partial aromatisation are the predominant modes of reaction of cholesterol with the melt.

From our results on cholesterol and other model systems, the following process apparently takes place when Green River kerogen is placed in a basic tetrachloroaluminate melt. Initially, hydrogen cations form which subsequently recombine with excess chloride, producing hydrogen chloride gas which escapes from the melt. The kerogen radicals formed can abstract labile hydrogen from another part of the kerogen macromolecule. These recombinations continue as free hydrogen cations and recombine with chloride resulting in an increase in the aromatic character while the macromolecule's size reduces from the various splittings which can occur. We believe it is this mechanism involving a mode of free radical intramolecular disproportionation combined with the ability of the melt to permeate the inorganic matrix of the shale which results in the ability to extract soluble organic material from Green River kerogen.

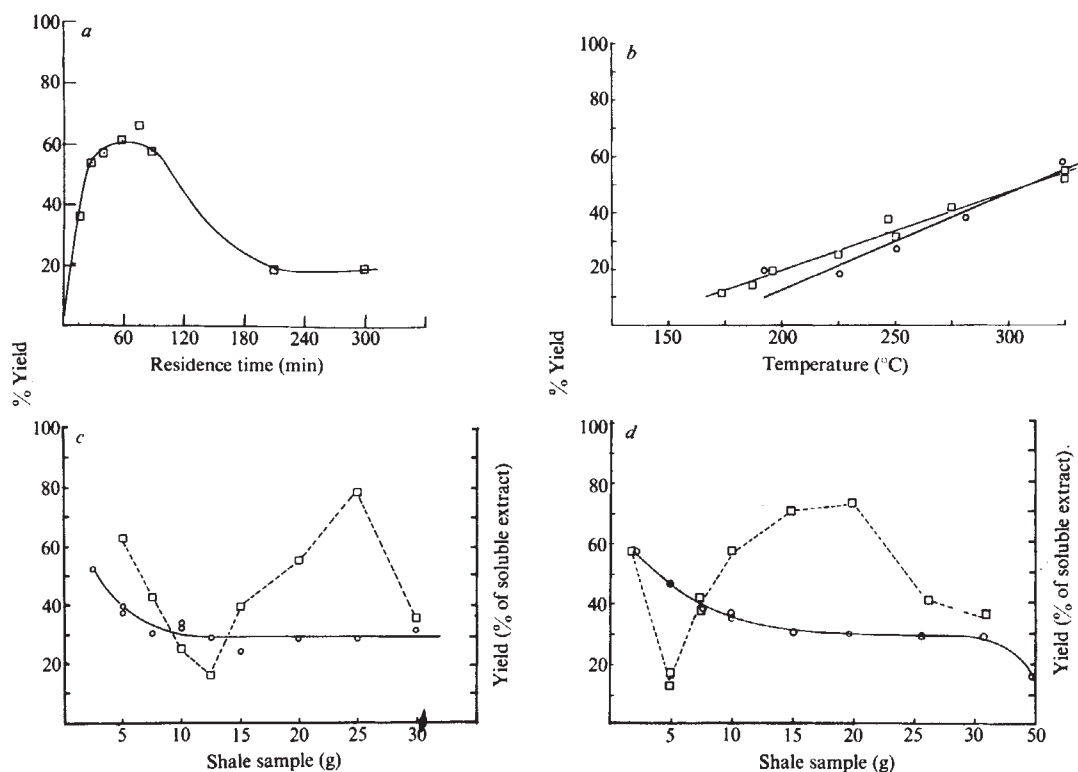


Fig. 2 a, Yield of soluble material as a function of time at 320 °C, 2.5 g sample. b, Yield of soluble material as a function of temperature 25 min, 2.5 g sample; □, unheated shale; ○, carbonate-free concentrate. c, Yield of soluble material as a function of sample, 25 min, 320 °C, 0.375 mol solvent; ○, unheated shale; □, benzene soluble material. d, Yield of soluble material as a function of sample, 25 min, 320 °C, 375 mol solvent; ○, carbonate-free concentrate; □, benzene soluble material.

This study demonstrates a possible oil-shale degradation process in the low temperature area of kerogen. But there are still many unanswered questions concerning both the nature and possible processing methods of oil shale.

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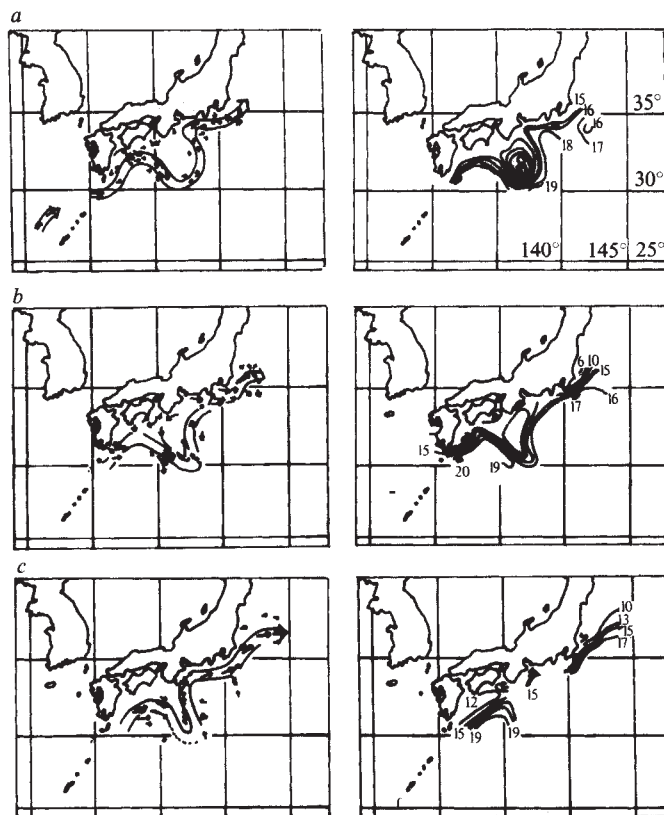


Fig. 2 Surface currents and 200-m isotherms during *a*, 2–14 February; *b*, 1–19 March; and *c*, 18–31 March 1977, before the detachment of the current ring (from Kaiyo Sokuho, Maritime Safety Agency, Tokyo). For clarity some GEK current vectors have been omitted, where the current was weak or the density of observations high, as have some isotherms.

Detachment and recombination of a current ring with the Kuroshio

A CURRENT RING detached from the large quasi-steady Kuroshio meander south of Honshu, for the first time in recorded oceanographic history, in May 1977, and its course is described here. The ring remained stationary until August, when it recombined with the main Kuroshio to reform a meander distorted in shape and upstream from the original meander.

The Kuroshio south of Japan is noted for its bimodal structure of paths. The so-called 'normal' path flows near the coast, as shown in Fig. 1. From time to time it flows in a large

meander, leaving the coast near Shiono Misaki and flowing as far south as 30° N on occasion before turning northwards and returning to the Japanese coast along the Izu–Ogasawara Ridge. The distribution of the paths of the current within the meander is bimodal¹. Meanders in 1934–44, 1953–56, and 1959–63 have been well documented in modern oceanographic observations²; an existing meander has been found which began in 1975. Previous meanders in 1870–75, 1890–91,

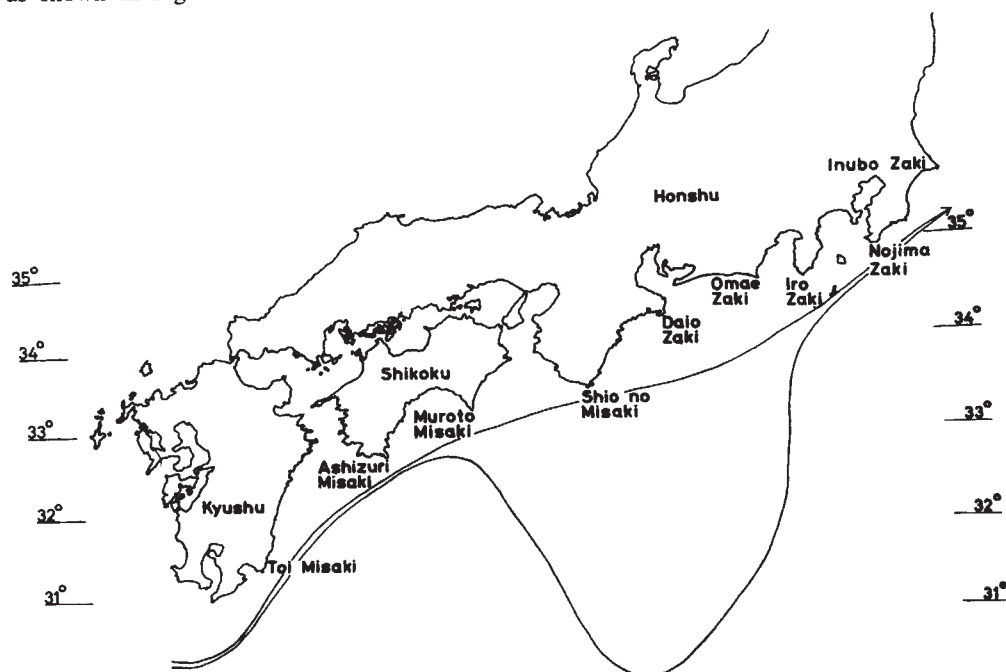


Fig. 1 Normal and meander paths of the Kuroshio south of Honshu.

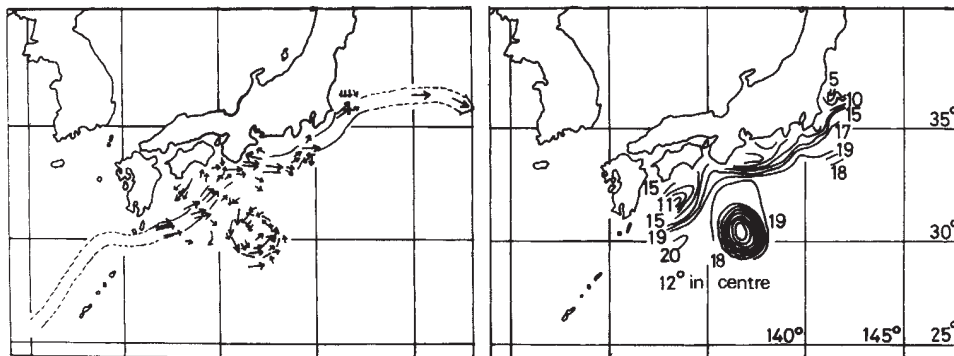


Fig. 3 Surface currents and 200-m isotherms during 1–14 July 1977, during the independent existence of the current ring.

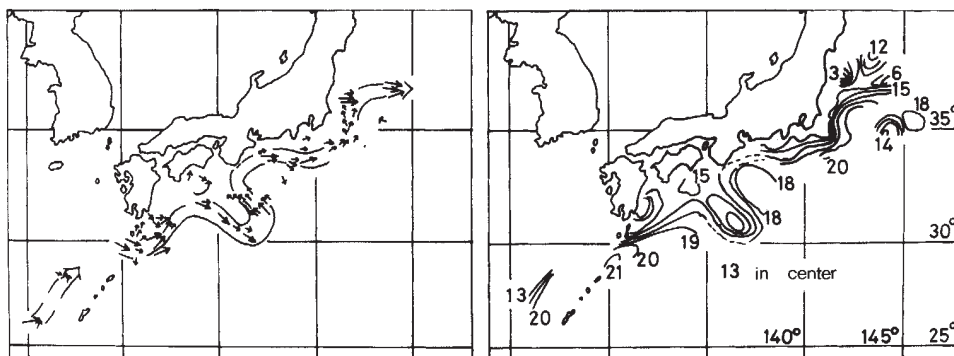


Fig. 4 Surface currents and 200-m isotherms during 1–16 August 1977, following recombination of the meander with the main Kuroshio.

1906–07, 1917–19 and 1925–28 have been inferred from evidence such as fishermen's records or relatively sparse oceanographic observations³. Smaller and/or transient meanders of the Kuroshio also frequently occur in this region.

The state of the Kuroshio can be followed through the twice-monthly reports of oceanographic conditions (Kaiyo Sokuho) issued by the Maritime Safety Agency in Tokyo. These are a compilation and interpretation of 0-m, 100-m, and 200-m temperatures and GEK observations, using data obtained from the regularly repeated sections of the Hydrographic Office and the Japan Meteorological Agency, and from naval and other ships.

The path of the Kuroshio is best indicated by the surface currents inferred from GEK measurements, and by the 200-m isotherms⁴. Figure 2a shows the surface currents and isotherms during the latter half of February 1977. This is a typical meander path, the current leaving the coast off Muroto Misaki, and reaching 30°N before turning northwards just west of the Izu–Ogasawara Ridge and returning to the coast between Daio Zaki and Omae Zaki. It then flows eastwards along the coast until the permanent separation off Inubo Zaki.

By mid-May the meander seemed to have become squeezed into an unusual, long and narrow shape extending SSE from Shio no Misaki (Fig. 2c). By the end of May a separate ring seemed to have broken off. The meander of the contiguous Kuroshio gradually disappeared and, by July, finally disappeared altogether, with the main current flowing straight past the coast at Shio no Misaki and the apparently enlarged eddy on its offshore side (Fig. 3). Finally, in August the main Kuroshio combined with the ring to reform the large meander (Fig. 4), but the meander was now in a modified path, to the west of its position before the ring formed.

Examples of recombination of a current ring with the Gulf Stream have previously been observed by Cheney *et al.*⁵, Richardson *et al.*⁶, and by D. R. Watts (personal communication). Those observed by Cheney *et al.* and by Richardson *et al.* drifted eastwards before coalescing with the Gulf Stream. Richardson *et al.* pointed out that this contradicts existing theories of current ring dynamics, so that the present Kuroshio ring may be less in contradiction to theory than the Gulf Stream rings. The coalescences reported by Cheney *et al.* and by Watts both resulted in S-shaped current paths

similar to that observed after the coalescence of the Kuroshio ring.

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Anomalous magnetic fabric in sediments which record an apparent geomagnetic field excursion

THE discussions of late Quaternary magnetic stratigraphy have presented a complex variety of magnetic events and excursions, many with differing degrees of reliability^{1,2}. Several authors^{1–4} have warned against this 'reinforcement syndrome' in interpreting unconfirmed palaeomagnetic results. In support of these admonitions, we report here that some apparent geomagnetic field excursions recorded in sediments may not represent short-term changes in the Earth's magnetic field.

Magnetic measurements of Core V from Imuruk Lake (65.6°N, 196.8°E), Alaska, result in a continuous record at a single site of geomagnetic field variation for a period >10⁵ yr. The lower half of Imuruk Core V, collected within 1 m of Core IV (ref. 5), yields a remanent magnetic (RM) inclination record (Fig. 1) which has apparently recorded at least one excursion of

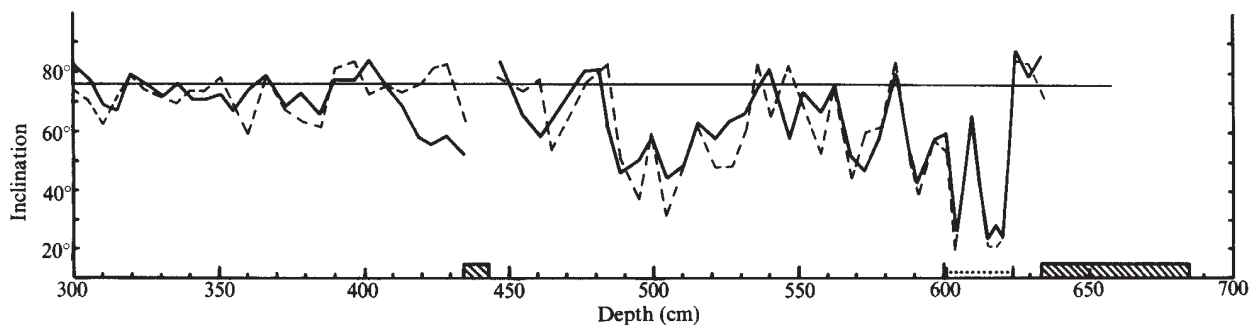


Fig. 1 Remanent magnetic (RM) inclination data for Imuruk Lake Core V (depth interval 300–684.7 cm). NRM, solid jagged line; dashed line, RM data after A.F. demagnetisation at 3.6×10^4 A/M. Hatched bars represent material which is unsuitable for sampling due to extreme desiccation. The dotted line brackets the interval of an apparent geomagnetic field excursion. Solid vertical line represents the inclination value of the Earth's magnetic field at the site⁵.

the geomagnetic field. Several zones of low inclination including at least one two-part RM inclination-shallowing of 55° occur near the base of Core V. A $>50^\circ$ inclination shallowing is, according to Noltimier and Colinvaux⁵, large enough to be considered as an 'excursion' at this site. Such shallow RM inclination values ($>50^\circ$) occur between 601 and 624 cm depth in the core and yield extrapolated age estimates, based on radiocarbon dates from Core IV, which nearly coincide with ages reported for the Blake magnetic polarity event^{6–9}. In addition, the bipartite nature of the inclination variation closely resembles Blake-event data from Fukushima Prefecture, Japan^{9,10} and the Greater Antilles Outer Ridge^{11,12}. It might be concluded from these data that a geomagnetic field excursion (the Blake Event) has been recorded in Imuruk Core V sediments. To test this conclusion, we have measured the magnetic

fabric of excursion and normal sediments in the core using the magnetic susceptibility anisotropy method.

Measurement of the anisotropy of magnetic susceptibility (AMS) may be the key to our understanding the magnetisation processes in sediments¹³. Certainly, AMS can be a sensitive indicator of primary or secondary sedimentary fabric^{14,15} and bottom-current-controlled deposition¹⁶. The AMS of 12 samples from Imuruk Core V has been measured using an automated low-field torque magnetometer¹⁷. Samples include six which define the hypothesised excursion near the base of Core V, and six other normal samples which were chosen at arbitrary intervals from those portions of the core which gave an RM inclination consistent with that expected for the site ($I = 70^\circ \pm 10^\circ$). Normal sediments exhibit a characteristic primary magnetic fabric (Fig. 2a), whereas excursion sediments exhibit an anomalous, distorted fabric (Fig. 2b). Several causes for the distorted magnetic fabric recorded in the excursion sediments, such as slumping, deposition by turbidity currents, secondary mineralisation, increases in biogenic activity, and other processes, are possible. The main consideration, however, is that the excursion magnetic fabric is anomalous, and therefore the magnetic directions recorded in Core V are suspect. We suggest that without independent corroborative evidence, such excursions should be regarded with suspicion.

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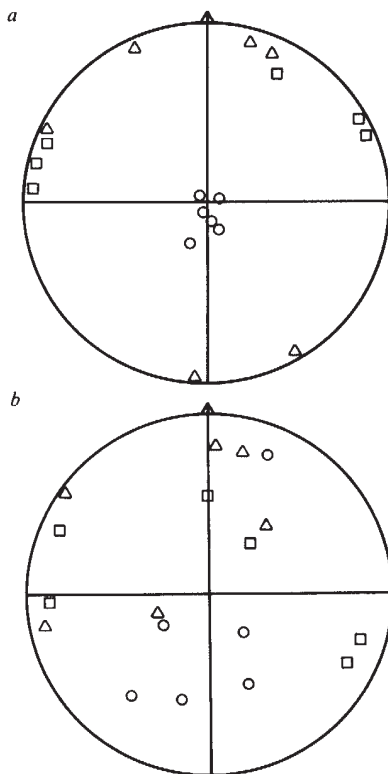


Fig. 2 Equal area projections of the magnetic fabric in samples from Lake Imuruk Core V. Both plots are relative to an arbitrary azimuth (arrow) and points represent upper hemisphere projections. a, Normal sediments were sampled at random intervals in the core from samples with RM inclinations $= 70^\circ \pm 10^\circ$, and exhibit characteristic primary fabric with well grouped, nearly vertical minima. b, Excursion sediments, sampled between 601 and 625 cm depth, represent random orientations characteristic of disturbed fabric. □, Maxima; Δ, intermediate; ○, minima.

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A horned beetle which fights

HORNS, and other often elaborate forms of prothoracic and cephalic armature, are a well known feature of male and occasionally female beetles in the family Scarabaeidae (Coleoptera)¹. Many of these, including rhinoceros beetles (Dynastinae) and dung beetles (Scarabaeinae, Geotrupinae), have been studied in detail, but the function of the horns is still the subject of dispute². The idea that in certain circumstances it would be an advantage for male insects to fight for the possession of a female or a breeding site, and that horns and similar appendages have evolved as weapons, has recently been put forward by Hamilton³. The male of the geotrupine *Typhoeus typhoeus* (L.) is well armed in this way (Fig. 1), and the observations that I report here show for the first time in a burrowing beetle, not only that the horns are used for fighting between males, but that they are specifically designed for the purpose.

T. typhoeus is found in sandy areas of Britain and Europe where it feeds principally on rabbit, sheep or deer droppings⁴, and it is common at Silwood Park where this work was carried out. Individuals vary greatly in size (Table 1). Differences between males are exaggerated by the horns, the length of which increases disproportionately (allometrically) with body length. Each male has three horns on its prothorax—two long and scimitar-like on the outside and one short and pointed on the inside. Adults emerge in late September, form pairs, and cooperate in digging breeding burrows of approximately 0.5–1.5 m in depth. At the bottom of the burrow, up to 10 sausages of compressed dung are formed for the developing larvae, one for each egg, and each taking about 24 h to complete (Fig. 2). Labour is divided between the pair, with the male uppermost in the burrow, removing soil and collecting dung pellets, while the female digs and constructs the sausages. Despite these activities, the male spends comparatively little time on the surface.

Outside its burrow, the male is obviously aggressive, much more so than the female. When prodded, he responds by stridulating loudly (the mechanism is found in the hind coxal cavity of both sexes). This is accompanied by powerful upward jerks of the head and prothorax, and extension of the prothoracic legs (an exaggerated version of normal burrowing movements). However, two males do not react to one another in this situation, and the necessary observations must be made within the burrow itself.

Behaviour 'underground' was first recorded using an artificial burrow cast in plaster-of-Paris. The open sides of the burrow were covered with 3-mm nylon mesh, leaving the beetle free to move inside, and allowing observation and interference from the outside. However, no breeding activity was possible. A lone male can be stimulated to defend a burrow of this kind by introducing another male, or by repeated poking through the mesh. The response to both of these takes two forms. If either beetle offers little or no resistance, the two beetles may face each other head-on, one pushing the other

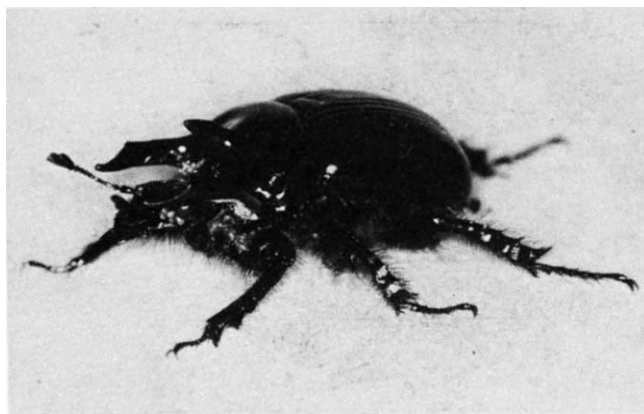


Fig. 1 Male *T. typhoeus* (L.) (photo: Frank Wright) (×3).

along the burrow, sometimes with the upward jerks already described (the 'direct push') (Fig. 3a). If the intruder presses his attack, the defendant turns on his side across the line of the burrow, with prothorax and elytra orientated towards the point of attack, and wedges himself in this position with his legs (the 'defensive block') (Fig. 3b). Now completely secure against any form of direct pushing, which is actively resisted, the beetle can only be dislodged by levering under the lower edge of his elytra. Figure 3b shows how the horns of the attacker combine with upward jerking of his prothorax to do this, if the attacker is large enough. If not, and his horns are too short, they scrape ineffectively on the defendant's shiny elytra. The defensive block is likely to be effective against males of a similar size or smaller, and against intruders of other species.

The more natural circumstances of an active breeding burrow can be provided in a soil-filled, glass-sided observation cage of the type described by Main⁵. The breeding activities of a pair of beetles can be recorded, but the two glass sides constrict the burrow, prevent a beetle from turning across it, and thus preclude the defensive block. A defending beetle will attempt to form a block by orientating itself vertically instead of horizontally, but this is also unsuccessful because the glass affords no grip.

Within the confines of a breeding burrow in a Main cage, the contest between two males usually takes place in the horizontal

Fig. 2 *Typhoeus* breeding burrow (vertical section).

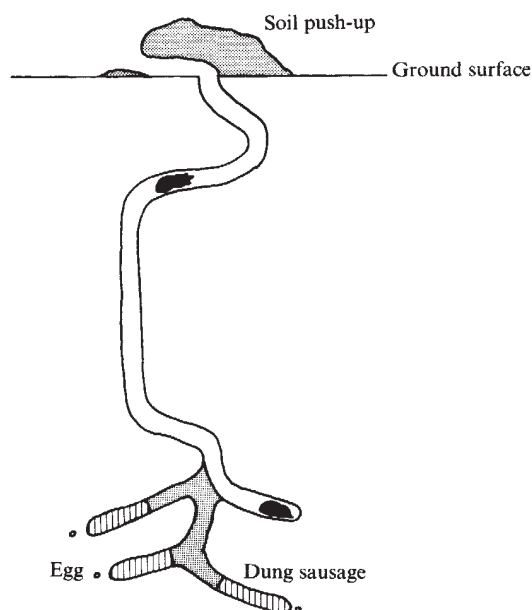


Table 1 Physical characteristics of *Typhoeus* males

Male	Live weight (mg)	Body length* (mm)	Horn length† (mm)
1	360	12.0	2.5
2	400	13.5	2.5
3	430	13.0	3.0
4	470	13.5	3.0
5	500	13.5	3.0
6	550	14.5	4.0
7	650	15.0	6.5

* Anterior edge of pronotum to posterior edge of elytra.

† Outer horn: tip to insertion along upper edge.

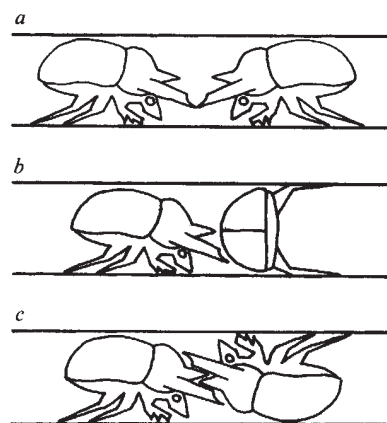


Fig. 3 Components of inter-male fighting within the burrow. *a*, Direct push; *b*, defensive block, with the defendant viewed end-on; *c*, pressing contest.

section just below the surface (Fig. 2). Two main components are observed. The first is the direct push already described (Fig. 3*a*), which usually appears after one of the males has established his supremacy. More important is the 'pressing contest' (Fig. 3*c*). As soon as the occupying male offers any resistance to an attacker, the two beetles meet head to head, one turns on his back, and the horns of each engage on the prothorax of the other. Both beetles jerk upwards against one another, stridulating continuously. In this position, neither male can push past the other, because of the pressure that they mutually exert, and the way in which a beetle's small central horn engages on the front of the prothorax of his opponent.

Each bout in a pressing contest lasts for not more than three minutes, with frequent lulls in activity, and ends when the beetles relax and break off, either by pushing past one another, or by turning away and retreating along the burrow. When the beetles are of nearly equal size, there may be several bouts, and the relative positions of the two in the burrow may change more than once in a contest lasting for up to 75 min. The pressing contest seems to establish which male is the stronger, and the loser either turns and leaves the burrow, or is pushed out by the winner (the direct push). Observations suggest that the heavier of the two males is always the winner, regardless of which was the original occupant (Table 2). Throughout the contest, the female seems quite indifferent to what is happening, and attempts to continue with her normal breeding activities. As soon as the contest is finished, even before the loser has left, the winner starts removing loose soil from the burrow, and a new occupant will copulate with the female within a few minutes.

Females have been observed using the defensive block and the direct push against males or other females, but without any signs of a contest developing. Pre-copulatory activity bears a resemblance to an inter-male contest, but the female shows no real aggression towards the male, who is never expelled from the burrow. Breeding activities start immediately after copulation.

Table 2 Contest results

Occupant*	Intruder	Winner
4	6	6
6	7	7
7	1	7
1	3	3
3	2	3
3	6	6
6	4	6
6	7	7

* Numbers as in Table 1.

In conclusion, it seems that the horns on the prothorax of a male *Typhoeus* have evolved as a means to gain purchase on the body of an opponent, facilitating a trial of strength using their powerful burrowing muscles in the confines of a burrow, and as a counter-measure to an otherwise impregnable position, the defensive block.

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Clonal segregation and positional information in late ommatidial development in *Drosophila*

CELLULAR DIVERSITY in an organ might arise from differential mitoses within a prefixed, genetically determined cell lineage or through the reading of positional values by equipotent cells in a morphogenetic field¹. We have studied this problem in the developing eye imaginal disc of *Drosophila*, in particular in the cells giving rise to the eight photoreceptor (R) and the five secondary pigment (SP) cells of the adult ommatidium^{2,3}. These cells lie close together within the ommatidium and are easily distinguishable with morphological criteria. We asked the following question: do R and SP cells have common precursors at the latest developmental stage or do they belong to different cell lineages?

One approach to this problem is the use of clonal analysis. Single eye anlage cells are genetically labelled with X-ray-induced mitotic recombination during the third larval instar, when the ommatidial cells are undergoing their final divisions. A cell autonomous marker², that is, one expressed in each mutant cell irrespective of phenotype of surrounding cells (white, *w* 1-1, 5), is used that affects pigmentation of both R and SP cells, and the composition of resulting spots is studied with histological techniques. (Questions about the interpretation of third instar spots are discussed in ref. 3.) If the spots contain both R and SP cells then there is no segregation of cell lineages; if the spots are confined to either cell type, however, there may exist a differential division segregating R from SP cell lineages. Nevertheless, the number of imponderables involved in clonal analysis at this level of resolution is very high. Thus the evidence from the analysis cannot be taken as conclusive, but only as indicative, for any one of the two determinative mechanisms under discussion. For instance, cells are only susceptible to mitotic recombination at a particular stage of their cell cycle; thus any local synchrony in the cycle of disc cells would obscure the results by increasing the probability for labelling two or more neighbouring anlage cells simultaneously. As a consequence the progeny of two neighbouring labelled cells will intermingle with each other and, since both clones carry the same marker, a distinction between them would be impossible³.

Table 1 shows the classes of spots collected in 24 compound eyes of *w*/+ flies irradiated as third instar larvae with 1,000 rad (100 Kv, 15 mA, 0.7 mm Al filter) (experiment 1). This dose was chosen to give a relatively high yield of spots. Spots are defined according to topological criteria discussed in ref. 3.

Mixed populations of R and SP cells were present in 11.2% of all spots. We have performed two different experiments to clarify the nature of these crucial mixed spots. The first (experiment 2) was similar to experiment 1 but the X-ray doses were reduced by half. As there is a linear dose relationship for single cell labelling^{4,5} the probability for simultaneous labelling of two neighbouring cells should have a square relationship. Therefore, decreasing the X-ray dose by half should result in a reduction to one-fourth of the probability for simultaneous double hits. Assuming that segregation of lineages takes place, the percentage of mixed spots should decrease considerably. Conversely, if the mixed spots actually reflect non-segregation of lineages rather than simultaneous hits in different neighbouring precursors, decreasing the X-ray dose should have no significant effect on the percentage of mixed spots.

Irradiating with 500 rad, without changing the remaining experimental conditions, the percentage of mixed spots is 3.1% (see Table 1), that is, a reduction of mixed spot frequency to one sixth as compared with the material irradiated with 1,000 rad (see Fig. 2). This result indicates that mixed spots are due to double events in neighbouring anlage cells.

The second test gives a direct estimate of the frequency of simultaneous hits to be expected on two different but neighbouring precursor cells. In this case we used markers on different chromosomes (retinal degeneration B, *rdgB* 1-42.7 and brown, *bw* 2-104.5) (experiment 3a). These markers express themselves exclusively in one or other of the two cell types under consideration: *rdgB* produces degeneration of adult R cells^{6,7} and *bw* affects pteridines⁸ that are only present in pigment cells. Both markers are autonomous in their expression^{2,6} (unpublished data). In this experiment a spot (defined using the same criteria above) that contains R and SP cells must have arisen from two independent labelling events, one in the first and one in the second chromosome. The results (Table 1) show that 13% of the spots are mixed (see Fig. 1)—the same proportion of spot mixing as that found for *w* spots in experiment 1.

This simultaneous labelling of R and SP cells in a high number of spots may be the result of having hit chromosomes in two different precursors, but it could also be the consequence of double events within the same cell. Given appropriate segregation of chromosomes at mitosis and no separate lineages of R and SP cells, the result would be clones containing both cell types⁵.

To estimate the frequency of simultaneous events in two chromosomes of the same cell, an experiment (experiment 3b) was performed using markers in different chromosomes but expressing themselves in the same cell type, *rdgB* and scarlet (*st* 3-44)⁸ that autonomously affects the pigmentation of R cells (data not shown). In this case any spot with *rdgB;st* double homozygous cells must have arisen from hitting the first and the third chromosome of the same cell. Among 24 compound eyes of *rdgB/+; st/+* flies irradiated with 1,000 rad as third instar larvae, 163 *rdgB* spots were collected, 20 of them associated

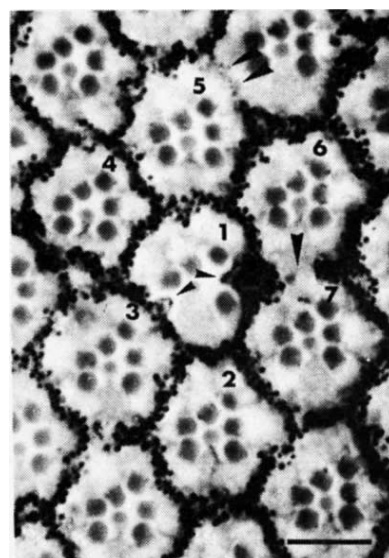


Fig. 1 Cross-section through a *rdgB/+; bw/+* compound eye from experiment 3a. Seven ommatidia have been numbered from 1 to 7. Ommatidium 1 has two *rdgB* R cells (small arrow-heads) which have been phagocytosed by SP cells. In ommatidium 5 one *bw* SP cell is marked with two large arrow-heads. According to the criteria discussed in ref. 3, all labelled cells contained within an area of seven contiguous ommatidia are operationally considered to belong to one spot. Thus *rdgB* and *bw* cells in this figure are taken to be members of the same spot. The large arrow-head in ommatidium 6 points to the site of a SP cell missing due to X-ray damage. Sites of missing SP cells can be clearly distinguished from *bw* SP cells because the former show a blank space whereas the latter still contain ommochromes which are visible in light microscope preparations. Twenty-four compound eyes have been histologically studied for each one of the experiments reported here, using the electron microscope in experiments 1, 2 and 3b and, since the phenotype of both markers is distinguishable on 2- μ m sections, the light microscope in experiment 3a. See ref. 3 for further details. Scale bar, 5 μ m.

with *st* spots. In no case were cells doubly labelled. Therefore the frequency of double hits affecting different chromosomes within the same cell must be very low.

The reason for the disparity in the frequency obtained for double events within the same and among different cells can be explained on the basis of local synchrony of dividing anlage cells. A simple estimation of the probabilities for these two phenomena, using the data of experiments 1 and 3a, has shown that 500 synchronised cells per disc, clustered in groups of about 10 cells each, would be sufficient to account for the results. In these circumstances the number of targets (chromosomes) for genetic labelling is greatly increased within a small territory. This assumption receives support from third larval instar ³H-thymidine autoradiograms³.

Table 1 Effect of X-ray irradiation on history of the ommatidium

				<i>w/+</i>					<i>rdgB/+; bw/+</i>		
				1,000 rad			500 rad		$\frac{500 \text{ rad}}{1,000 \text{ rad}}$ ratio		1,000 rad
Composition of spots	<i>n</i> *	%	Size†	<i>n</i>	%	Size	<i>n</i>	Size	<i>n</i>	%	Size
RC	145	65.0	1.48±0.72	90	69.8	1.28±0.64	0.62	0.86	135	62.5	1.45±0.79
SPC	53	23.8	1.72±0.82	35	27.1	1.17±0.38	0.66	0.68	53	24.5	1.17±0.55
RC+SPC	25	11.2	4.6 ±2.2	4	3.1	3.50±2.38	0.16	0.76	28	13.0	2.75±0.96
Total	223	100.0	1.88±1.4	129	100.0	1.31±0.78	0.58	0.69	216	100.0	1.56±0.92

*No. of spots among 24 compound eyes.

†Cells per spot. Values are means ± s.d.

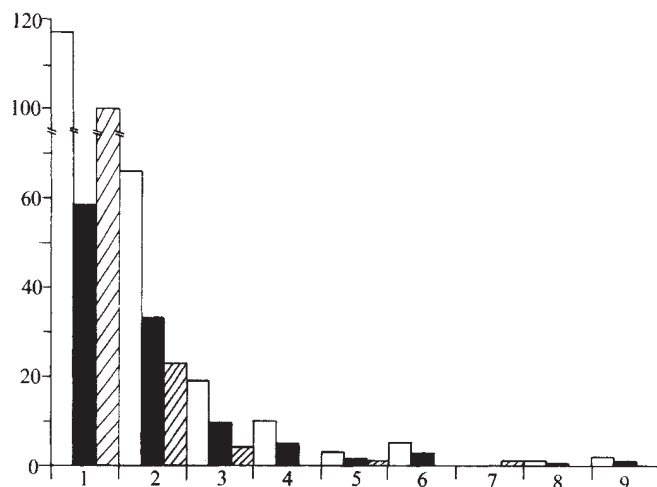


Fig. 2 Spot distribution according to size classes (in cells), from experiment 1 (open bar) and experiment 2 (cross-hatched bar). Values in solid column result from dividing by 2 those of experiment 1. This distribution of spot size is to be expected when irradiating with 500 rad, provided that no double labelling events take place. However, the actual values obtained from experiment 2 significantly differ from the predicted ones ($P < 0.001$).

Our results clearly show that frequent simultaneous labelling events take place in different cells that will differentiate after division(s), as mixed spots of R and SP cells. This leads to the assumption that similar double events should also occur between different cells that will differentiate either as R or as SP cells; that is, pure R and pure SP spots in experiment 1 are expected to derive from two simultaneously labelled, neighbour anlage cells in a similar frequency as that found for mixed spots. In fact when irradiating with 500 rad (experiment 2) both a decrease of spot size and an increase of the proportion of spots containing a single cell were observed—as expected, if some of those with two or more cells from experiment 1 are due to double events (Table 1 and Fig. 2).

The decrease of mixed spot frequency with reduced X-ray dose (experiment 2) and the high frequency of simultaneous labelling events within different cells (experiment 3) strongly suggest that the mixed spots in experiment 1 are due to the same mechanism, namely a simultaneous labelling of R and SP cell precursors. These experiments and results do not clearly demonstrate the clonal segregation of cell lineages, but the results of the clonal analysis reported here are consistent with this hypothesis.

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Induction and separation of antigen-dependent T helper and T suppressor cells in man

INDUCTION of plaque-forming cells (PFC), specific for sheep red blood cells (SRBC) or ovalbumin (OA), from human peripheral blood requires the cooperation of surface IgM-positive B cells and E-rosette-forming T lymphocytes^{1,2}. At high antigen concentrations PFC responses are suppressed² and it seemed that this effect might be mediated in part by antigen-stimulated T lymphocytes^{2,3}. We have studied the functional activity of purified T cells which had been pre-incubated ('primed') for 24 h in the presence of a high concentration of antigen. We report here that such primed cell preparations contain antigen-specific suppressor cells which interfere with the induction of PFC in autologous target PFC cultures. These suppressor cells and their precursors could be isolated in the theophylline-sensitive lymphocyte fraction⁴.

Primed cells were added to target PFC cultures containing autologous peripheral blood mononuclear cells (PBL) or purified B lymphocytes and a non-suppressive concentration of antigen ($1 \mu\text{g OA ml}^{-1}$). In addition to purified T lymphocytes, T cell subpopulations were isolated and tested in a similar fashion. These populations were obtained in a procedure which takes advantage of the effects of the drug theophylline, which reversibly inhibits the ability of a distinct T cell subset to form E rosettes⁴: these 'theophylline-sensitive' (T-sens) T lymphocytes were separated from a population (of roughly similar size) of 'theophylline-resistant' (T-res) lymphocytes by E rosette depletion of purified T cells in the presence of the drug. Theophylline-resistant E-rosette-forming cells sedimented as lymphocyte-SRBC complexes through Ficoll-Hypaque gradients. T-sens cells, on the other hand, having lost the ability to form E rosettes, remained at the interface of the gradients.

The effect and specificity of primed T cells were analysed using target PFC cultures of 3×10^6 fresh PBL (Table 1). For antigen priming, 5×10^6 T cells, purified by E rosette depletion of PBL, were incubated for 24 h in 5 ml RPMI-1640 containing $50 \mu\text{M}$ 2-mercaptoethanol, 10% human serum and $100 \mu\text{g}$ OA. In control cultures human or horse albumin were used instead of OA. Washed primed T cells were then added (1×10^6 per tube) to target PFC cultures containing $1 \mu\text{g ml}^{-1}$ OA (see Table 1). Aliquots of these primed T cells were separated into T-sens and T-res cells and added to individual target PFC cultures. The numbers of OA-specific PFC generated were assessed by standard procedures after 5 d of culture¹.

OA-specific PFC responses in PBL cultures containing no additional T cells or 1×10^6 human or horse albumin-primed T cells ranged from 2 to 2.5×10^3 PFC per culture (Table 1). In contrast, the addition of OA-primed T cells resulted in significant, antigen-specific suppression of the PFC response (1×10^3 PFC per culture). To characterise the population mediating this suppressive effect, OA-primed T cells were separated into T-sens and T-res cell populations. In these experiments the addition of primed T-sens cells resulted in almost complete suppression of the PFC response, whereas addition of similarly primed T-res cells resulted in a marked enhancement of PFC generation.

In an attempt to further characterise these T cell subsets, we correlated functional activity with cell surface markers. Moretta *et al.*⁵ have distinguished T helper and T suppressor activities in man, demonstrating receptors for the Fc portion of IgM (T_H) on helper cells and receptors for the Fc portion of IgG (T_S) on mitogen-induced suppressor cells. We have shown that the majority of T-sens cells carried receptors for IgG, whereas IgM receptors were found on T-res cells (Table 1). Using IgM- or IgG-coated ox red blood cells in a rosette

depletion procedure, we isolated the $T\mu$ and $T\gamma$ subsets contained in the OA-primed purified T cell preparation and assayed their activity as before (Table 1). Helper cell activity seemed to be restricted to the $T\mu$ -enriched populations, whereas suppressor cells were found in the $T\gamma$ -enriched preparations. These studies indicated that in the presence of high antigen concentration, both helper and suppressor activities are generated concomitantly and the cell populations mediating these activities can be separated on the basis of Fc receptors as well as by the use of the drug theophylline. Moreover, the use of theophylline in the separation of antigen specific suppressor cells circumvents the suggested requirement for activation of suppressor cells by immune complexes⁵.

To characterise the direct precursor cells of helper and suppressor cells as well as requirements for their induction, the previous protocol was maintained with the exception that T-res and T-sens cells were isolated from purified T cells before antigen priming (Table 2). 1×10^6 primed T cells were then added to target cultures of fresh PBL as before. The addition of OA-primed T cells or T-sens cells resulted in suppression of the PFC response in PBL. The degree of suppression was similar whether priming was carried out after (Table 2) or before cell separation (Table 1). The addition of T-res cells resulted in enhancement of the PFC response. Thus helper and suppressor cells were recruited from distinct and separable T cell pools and could develop under the same antigenic stimulus.

To minimise effects attributable to unprimed T cells in target PFC cultures of PBL, primed T cells were also added to target

Table 1 Separation of antigen-primed helper and suppressor T cell subpopulations

T cells added	Phenotype		Anti OA response (PFC per culture)
	$T\mu$ (%)	$T\gamma$ (%)	
None added			2,100 $\pm$ 220
Primed T cells			
Human albumin	53	17	2,000 $\pm$ 170
Horse albumin			2,450 $\pm$ 310
OA			1,030 $\pm$ 120
OA-primed T cell subpopulations			
T-sens	3	91	300 $\pm$ 110
T-res	86	5	3,800 $\pm$ 260
$T\gamma$ -enriched	5	81	420 $\pm$ 90
$T\gamma$ -depleted	74	6	3,300 $\pm$ 380
$T\mu$ -enriched	69	9	3,100 $\pm$ 310
$T\mu$ -depleted	17	49	1,200 $\pm$ 150

PBL (3×10^5 ml⁻¹) from healthy adult volunteers were obtained by density centrifugation on Ficoll-Hypaque gradients and cultured in upright 15-ml plastic tubes containing 10 ml medium RPMI-1640, 10% human serum, 50 μ M 2-mercaptoethanol and 1 μ g OA ml⁻¹. After 5 d of incubation, anti-OA PFC responses induced in these cultures in the presence or absence of 1×10^6 antigen-primed autologous T lymphocytes were measured in a haemolytic microplaque assay for direct PFC¹. Results are expressed as the means of triplicate determinations $\pm$ 1 s.d. of one such experiment. Results have been substantiated in four separate experiments using cells from different donors. For high dose antigen priming, 1×10^6 purified T cells ml⁻¹ were incubated for 24 h in 5 ml culture medium containing 100 μ g OA. Controls contained 100 μ g human or horse albumin. After 24 h these cells were washed and 1×10^6 cells were added to the 'target' cultures of fresh autologous PBL. Cell separations: a mixture (1:60) of lymphocytes and SRBC in phosphate buffered saline (PBS, pH 7.2) containing 20% fetal calf serum was incubated for 20 min at 8°C and then separated (30 min at 8°C, 1000 g) on Ficoll-Hypaque gradients. Lymphocyte-SRBC complexes (E rosettes) sedimented through the gradient and were used as a source of purified T cells. T-res cells were separated from T-sens cells in the same manner using purified T cells treated with 3 mM theophylline (60 min incubation at 37°C). Ox red blood cells coated with purified IgG or IgM and purified T cells were used for rosette depletions of $T\gamma$ and $T\mu$ lymphocytes⁵. The same complexes were used in rosette assays for the quantitation of $T\gamma$ or $T\mu$ cells in all cell preparations obtained.

Table 2 Identification of helper and suppressor cell precursors before antigen priming

T cells added	Anti-OA response (PFC per culture)	
	PBL	B lymphocytes
None added	2,100 $\pm$ 220	<200
Purified T cells	890 $\pm$ 130	650 $\pm$ 110
T-sens	520 $\pm$ 70	460 $\pm$ 80
T-res	3,700 $\pm$ 320	4,100 $\pm$ 290

T cell subpopulations were separated before high dose antigen priming. Purified T cells, T-sens cells or T-res cells were then primed for 24 h with 100 μ g OA per culture and washed; 1×10^6 cells were added to fresh autologous target cultures of 3×10^6 PBL. B cell target cultures contained 7×10^5 E-rosette-depleted PBL (<0.1% E-rosettes); this concentration provided for similar numbers of B lymphocytes per culture as present in PBL cultures.

PFC cultures of purified B cells. These B lymphocytes were obtained by E rosette depletion of autologous PBL and contained less than 0.1% E-rosette-forming cells. They failed to generate PFC in the absence of a T cell supplement. T-res cells provided helper activity for the generation of a high PFC response in cultures of B lymphocytes (column 2, Table 2). This response was maximal and could be enhanced only by the addition of B lymphocytes but not by the further addition of purified primed or unprimed T cells or T-res cells (data not shown). Thus, T-res, primed and cultured in the absence of additional T cells, could acquire and directly express helper activity. Cocultures of B lymphocytes and primed T-sens cells consistently developed a low but significant PFC response approaching the levels generated in cocultures with primed, purified T cells (which contained both helper (T-res) and suppressor (T-sens) cells). Thus T-sens cells could provide some helper activity to purified B cells, although they were suppressive to PBL target cultures. This may reflect incomplete depletion of helper activity from T-sens cells or it may reveal a requirement for the interaction of T-sens cells with primed (Table 1) or unprimed (Table 2) T-res cells for the development or expression of suppressor cell activity. The latter alternative would imply that B cells may not be the (sole) target of antigen-inducible suppressor T cells.

In summary, suppressor T lymphocytes, interfering with the generation of haemolytic PFC, were induced in populations of purified autologous T cells. Suppressor and helper cells and their direct precursors were isolated as T-sens and T-res, respectively, and seem to express mutually exclusive Fc receptors for either IgG or IgM. The generation of both activities occurs independently and concomitantly on exposure to high antigen concentration. The delineation of functionally distinct human T cell subpopulations with different surface receptor characteristics and drug reactivities now permits further analysis of the regulative events governing the expression of an immune response in man.

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Stage-specific antibody-dependent eosinophil-mediated destruction of *Trichinella spiralis*

THE nematode *Trichinella spiralis* provides a model in which host responses to helminth infection can be studied. In trichinosis, there are three phases against which host resistance might develop. First, resistance may be directed against adult worms in the gut, which develop there after inoculation of the host by infective larvae. Second, resistance may be directed against newborn larvae, which penetrate the gut mucosa and pass by way of the bloodstream to the skeletal muscles. Finally, it may be directed against larvae in the muscle cells, which mature in 2–3 weeks into infective third-stage larvae¹. In the study reported here we investigated the ability of white cells to kill each of the three stages of the life cycle of the parasite, that is, adult worms, newborn larvae, and muscle larvae. We describe here an antibody-dependent eosinophil-mediated destruction mechanism specific for the newborn larval stage of *T. spiralis*.

Infection in a colony of female CF1 mice (18–20 g; Carworth Farms, New City, New York) was established with *T. spiralis*. Muscle larvae, adult worms, and newborn larvae were prepared as described in Fig. 1. Peritoneal exudate cells were obtained from control uninfected and mice infected with *T. spiralis* for 1–6 weeks. To obtain eosinophil-rich peritoneal exudate cells from another source, CF1 mice infected with 50 cercariae of *Schistosoma mansoni* for 8 weeks were used. Pooled immune serum was obtained from mice infected with *T. spiralis* for 4–10 weeks.

Cells obtained from mice infected with *T. spiralis* for 5 weeks, in the presence of immune serum (cell:larva ratio 10,000:1), induced a mean of $32 \pm 10\%$ dead newborn larvae by 8 h, and increasing to $78 \pm 1\%$ at 24 h. No significant killing of larvae occurred in the presence of immune or normal serum, or with cells alone. We therefore decided to assess larval killing after 24 h of incubation in all subsequent experiments. Ninety-five per cent of newborn larvae were covered with more than 10 cells after 2 h. Cell attachment was not observed when normal serum was substituted for immune serum.

As the ratio of effector cells to newborn larvae was increased in the presence of immune serum, there was enhanced destruction of organisms at 24 h of incubation (Fig. 1). Decomplementation of immune serum by heat inactivation had no effect on cell-mediated damage of newborn larvae.

The effect of duration of infection with *T. spiralis* on the ability of white cells to kill newborn larvae is shown in Fig. 2. Cells obtained from mice infected for less than 4 weeks, when incubated with newborn larvae for 24 h, were incapable of mediating significant larval destruction. Peritoneal exudates of these animals contained approximately 70% macrophages, 28% lymphocytes, and less than 2% eosinophils. When peritoneal exudate cells were obtained from mice infected for 4–6 weeks (containing more than 20% eosinophils), up to 99% killing of newborn larvae was seen in the presence of immune serum. These results suggest that the eosinophil may be the major effector cell inducing larval killing. When we obtained eosinophil-rich (more than 25%) peritoneal exudates from *S. mansoni*-infected mice, significant killing ($P < 0.001$, *t* test) of *T. spiralis* newborn larvae was again observed in the presence of immune serum at all cell:larva ratios (for example, $53.0 \pm 7.1\%$ killing at a 10,000:1 cell:larva ratio). No such destruction was seen when cells were incubated in normal mouse serum ($4.7 \pm 2.9\%$ killing).

Confirmation of the role of the eosinophil as the major effector cell was obtained by two additional experiments. First, preincubation of eosinophil-rich peritoneal exudates with monospecific antieosinophil serum (AES)³ and complement

abrogated the cell-mediated destruction of newborn larvae ($79.9 \pm 4.2\%$ killing with control peritoneal exudates compared with $10.6 \pm 5.2\%$ killing with AES-treated peritoneal exudates). Second, eosinophil enrichment by density gradient centrifugation⁴ resulted in enhanced newborn larval killing, that is, $52.8 \pm 3.4\%$ larval killing compared with $4.5 \pm 8.4\%$ killing for the eosinophil-depleted fraction.

Further studies have confirmed that cellular attachment and larval destruction is mediated by an opsonic antibody. Antibody-coated newborn larvae were prepared by incubation of larvae in immune serum at 37°C for 2 h. After washing three times in medium, the larvae were incubated with peritoneal exudate cells from mice infected with *S. mansoni*; $76 \pm 7.2\%$ larval killing was observed at a 10,000:1 cell:larva ratio compared with $4.7 \pm 2.9\%$ killing when larvae were preincubated with normal mouse serum ($P < 0.001$, *t* test). Furthermore, when immune serum was absorbed by preincubation with newborn larvae and incubated with cells and newborn larvae, no statistically significant killing of organisms occurred ($16 \pm 4.8\%$).

The antibody-dependent cell-mediated damage of *T. spiralis* that we have described is specific for the newborn larva. Destruction of adult worms or muscle larvae (25 per well) was not observed at any cell: worm ratio, although adult worms and muscle larvae were covered with cells.

Destruction of newborn larvae may be one of the primary mechanisms of resistance in natural infection with *T. spiralis*. Although resistance is also directed against adult worms^{5,6}, and eosinophils attach to this stage *in vitro*⁷, some studies indicate that parenteral stages can induce, and are subject to, protective immunity^{8,9}. Newborn larvae are the smallest form of the parasite; unlike adult worms and muscle larvae they do not possess a thick and resistant cuticle¹. Furthermore, by being the only stage which migrates through the blood and extracellular

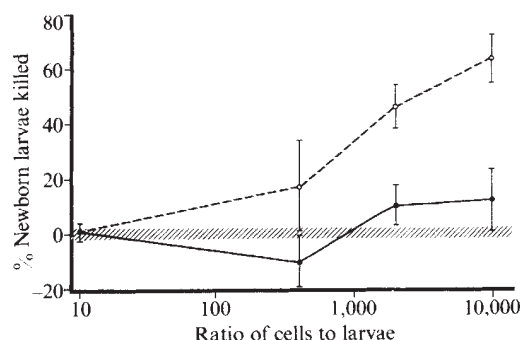


Fig. 1 Peritoneal exudate cells from mice infected with *T. spiralis* for 5 weeks were obtained 48 h after intraperitoneal injection of 1.5 ml 10% proteose-peptone. Adult worms were obtained from the small intestines of animals infected 7 d previously with 600 muscle larvae. To induce newborn larval production, 100–200 clean adult worms were incubated at 35°C for 24 h in 15 ml RPMI-1640, containing 30% heat-inactivated fetal calf serum (GIBCO) 100 IU penicillin and 100 μg streptomycin per ml (ref. 2). Newborn larvae were washed three times in 0.15 M NaCl and adjusted to 500 per ml supplemented RPMI-1640. Incubations in triplicate were carried out as follows: 0.1 ml serum, 0.1 ml cell suspension, and 0.1 ml *T. spiralis* worms or larvae suspension were added to microtitration plates with flat-bottomed wells (Falcon 3040), sealed, and incubated at 37°C for 24 h. ●, Normal serum; ○, immune serum. Control wells contained *T. spiralis* and normal or immune serum with no cells, or cells with no serum. The contents of each well were aspirated and the number of worms or larvae counted by light microscopy in Sedgwick-Rafter chambers (Curtin-Matheson). Cytotoxicity resulted in lysis of organisms; the percentage of killing was calculated as follows: (No. in control wells – No. in experimental wells)/(No. in control wells) $\times 100$. Significant killing of newborn larvae occurred only in the presence of immune serum. The percentage of newborn larvae destroyed increased in proportion to the cell:larva ratio.

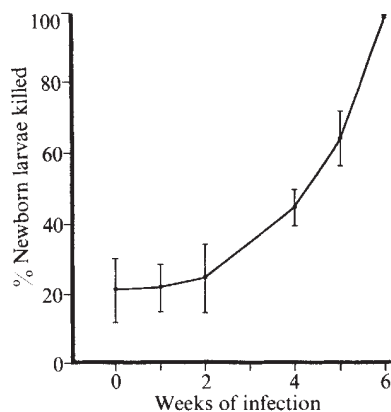


Fig. 2 Newborn larvae were incubated at a cell:larva ratio of 10,000:1 with cells obtained from peritoneal exudates of mice infected with *T. spiralis* for 0–6 weeks. The percentage ($\pm$ s.e.) of newborn larvae killed after incubation with immune serum for 24 h is shown. Statistically significant killing was first seen at 4 weeks.

tissues, they may be more susceptible to host defence mechanisms. *In vivo* studies have suggested that this resistance may be dependent on eosinophils, as we have shown previously that depletion of these cells increased the number of larvae in muscles, whereas it has no effect on adult worms in the gut¹⁰. The present *in vitro* studies confirm that the newborn larvae are susceptible to attack by eosinophils; the destruction requires the presence of specific antibody. This model of cell-mediated parasite destruction may provide a tool for investigation of the basic mechanisms whereby vertebrate hosts are able to combat infections with *T. spiralis* and other helminths.

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α_1 -Antitrypsin is an effector of immunological stasis

SEVERAL proteins in normal serum can serve as suppressors or enhancers of the immune response. Recently an α -globulin-rich fraction of Cohn fraction IV, designated IRA (immune-regulatory α -globulin), has been implicated in suppressing the *in vitro* antibody response of mouse spleen cells to sheep red cells without cytotoxicity¹. Studies by Chase² also indicate that α_2 -macroglobulin, one of the protease inhibitors present in normal serum, limits the human lymphocyte response to phytohaemagglutinin, and concanavalin A as measured by ³H-thymidine incorporation. A similar role for α_1 -antitrypsin (α_1 -AT), the major protease inhibitor in normal serum, can be

Table 1 Effect of α_1 -AT on anti-SRBC responses of mice

Treatment*	Dose (μ g per mouse)	Anti-SRBC responses† (PFC $\times 10^3$ per spleen)‡	
		Direct	Indirect
α_1 -AT	1,000	75 $\pm$ 4	2 $\pm$ 0.28
α_1 -AT	500	85 $\pm$ 11	3 $\pm$ 0.8
α_1 -AT	250	96 $\pm$ 5.2	9 $\pm$ 2.6
HSA	1,000	139 $\pm$ 7.4	17 $\pm$ 2.4
—	—	156 $\pm$ 16	19 $\pm$ 5.5

* Each mouse also received 10^7 SRBC intravenously.

† A 0.05% solution of agarose in minimal essential medium with Hanks base was distributed in 0.4 ml aliquots into pre-warmed Wassermann tubes at 53°C. 0.05 ml of one-fourth (v/v) SRBC suspension and spleen cells (0.1 ml) were added and the mixture then spread on a slide precoated with 0.1% agarose. After incubation for 1 h at 37°C in humid 8% CO₂ atmosphere, the slides were treated with 1:10 dilution of guinea pig serum as a source of complement. The slides were incubated for a further 2–3 h and PFC counted.

‡ Means $\pm$ s.e.m. of four mice.

proposed for involvement in the regulation of the immune response. Proteases have been found on both the surface and in the medium of cultured cells³; they have also been implicated in altering the electrophoretic mobility⁴, *in vivo* migration⁵ and blast transformation⁶ of lymphocytes. The physiological function of α_1 -AT in the serum is unknown. It is not clear why there is an increase of this protease inhibitor in certain physiological and pathological conditions⁷. Results presented here suggest that α_1 -AT has an immunoregulatory role, by suppressing the *in vivo* and *in vitro* immune response of mouse spleen cells against sheep red cells (SRBC).

Spleens from 9–16 week old hybrid C57BL/10 $\times$ C3H (BC3F₁) female mice were dissected free of the surrounding fascia and cells were expressed from the capsules. Single cell suspensions were gently aspirated and expelled by syringe through needles of 21 and 27 gauge. The cells were washed once then resuspended in spleen cell medium⁸. Viability was checked by the Trypan blue exclusion method.

α_1 -AT activity was determined by measurement of its trypsin inhibitory capacity⁹ and quantitatively confirmed by radial immunodiffusion¹⁰. It was also found to have a normal M phenotype pattern by isoelectric focusing¹¹ and by disc gel electrophoresis¹².

Immunoregulatory effects of protease inhibitors were studied *in vivo* by injecting various concentrations of α_1 -AT into groups of BCF₁ mice. α_1 -AT 250–1,000 μ g was injected intravenously together with 10^7 SRBC. The control groups received either human serum albumin (HSA), or human γ -globulin (HGG). Results presented in Table 1 indicate that these concentrations of α_1 -AT significantly suppressed the plaque response. Furthermore, as the dose of α_1 -AT was increased, greater suppression of plaque-forming cells (PFC) response resulted.

Table 2 Kinetics of α_1 -AT on *in vitro* anti-SRBC responses

Addition	Exposure time of culture* to α_1 -AT (days)	Anti-SRBC responses (PFC per culture)†
α_1 -AT	5	320 $\pm$ 43
α_1 -AT	4	395 $\pm$ 57
α_1 -AT	3	633 $\pm$ 41
α_1 -AT	2	841 $\pm$ 71
α_1 -AT	1	1,251 $\pm$ 120
—	—	1,229 $\pm$ 96

* Spleen cell cultures of 2×10^7 cells with SRBC.

† Mean $\pm$ standard error of 5 cultures.

Table 3 α_1 -AT effect on adherent and non-adherent spleen cells following antigen stimulation

Groups	Anti-SRBC responses (PFC per culture)
Adherent* + non-adherent†	564 ± 36
Adherent† + non-adherent*	161 ± 11
Adherent* + non-adherent*	33 ± 5
Adherent + non-adherent†	599 ± 50

Values are means ± s.e.m. of five cultures.

* α_1 -AT treatment.

† Untreated cells.

α_1 -AT effects on the immune response were studied *in vitro* by adding various amounts of the protease inhibitor to spleen cell cultures. 2×10^7 spleen cells ml^{-1} with 0.05 ml of 1.5% SRBC were cultured in Marbrook vessels at 37°C for 5 d at 8% CO_2 . The control group received either HSA, HGG, or an equivalent amount of medium. Haemolytic plaque assays for anti-SRBC producing cells (PFC) were conducted using a modified slide method of Mishell and Dutton¹³. At 100 μg per culture α_1 -AT significantly reduced the plaque response to 49% of the control. Increasing concentrations of α_1 -AT from 100 to 1,000 μg per culture resulted in even greater suppression of PFC responses (data not shown). α_1 -AT had no effect on plaque response at 1–50 μg per culture.

In control experiments HSA, HGG or acid glycoprotein fraction (prepared according to the method of Hao *et al.*) had no effect on spleen cell culture responses to SRBC. Also, α_1 -AT was shown not to bind to antigen, and not to lose biological activity in the culture conditions used. To exclude the possibility that α_1 -AT suppression was due to an effect on the antigen, SRBC were pretreated with α_1 -AT, washed and then added to the cultures. Results indicated that suppression by α_1 -AT was not due to an effect on the antigen.

Kinetic studies (Table 2) indicate that exposure to α_1 -AT for 48 h was sufficient to produce a reduction in plaque number; a further decrease was seen when spleen cells were exposed to α_1 -AT for up to 5 days. To investigate whether immunosuppression by α_1 -AT was a result of interaction with the adherent cell population (phagocytic cells) of spleen, groups of cultures were incubated with either α_1 -AT or an equivalent amount of culture medium. After 72 h, non-adherent cells were separated from adherent cells by repeated washing of the monolayers.

Combinations of treated and untreated adherent and non-adherent cell populations were arranged as shown in Table 3. Anti-SRBC responses of these cultures indicate that immunoregulation by α_1 -AT was not due to an effect on the adherent cells. To characterise the lymphoid cell type regulated

Table 4 Effect of α_1 -AT on T cells, B cells and adherent cells of spleen

Groups	Anti-SRBC responses† (PFC per culture)
T* + B + A	515 ± 13
T + B* + A	238 ± 19
T* + B + A*	380 ± 28
T + B* + A*	165 ± 9
T* + B* + A*	69 ± 18
T + B + A	552 ± 51

A, adherent cells of spleen.

* α_1 -AT treatment.

† Mean ± s.e.m. of five cultures. Cultures in the various groups consisted of 10^7 T cells and 10^7 B cells.

by α_1 -AT, groups of spleen cell cultures were set up as before. After separating the non-adherent cells by repeated washings of the monolayer, the non-adherent cells were further separated into T cells and B cells, by passage over nylon-wool columns. Combinations of T cells, B cells and adherent cells were made as described in Table 4. These studies indicate that α_1 -AT does not affect adherent cells or T cells. It does, however, regulate antigen-dependent B-cell responses.

Thus, although having no apparent effect on cell viability, α_1 -AT significantly inhibits the immune response both *in vitro* and *in vivo*. Work is in progress to further examine the mechanism of action of α_1 -AT. Of particular interest is its regulatory effect on cellular proteases. These studies should give information on the molecular events in lymphocyte maturation and the link between proteinase inhibitor and immunodeficiency.

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A common progenitor for human myeloid and lymphoid cells

THE pluripotent haemopoietic stem cell has been the object of many investigations in murine and human systems, but the origin of lymphoid cells from the human pluripotent stem cell has not been definitively established. In mice, investigations using the spleen colony technique¹ have demonstrated the existence of a pluripotent stem cell which can give rise to mixed colonies of erythrocytes and granulocytes². Further studies with radiation-induced chromosome markers in murine systems have provided evidence for the origin of lymphoid cells from this stem cell^{3–5}. The use of X-linked glucose-6-phosphate dehydrogenase (G-6-PD) isoenzymes as cell markers has provided another approach, applicable to the evaluation of human proliferative disorders^{6,7}. Those females with electrophoretically distinguishable G-6-PD isoenzymes who have clonal haematological disorders provide a source for such studies. Thus, Fialkow *et al.*⁸ have demonstrated the origin of erythrocytes, granulocytes, megakaryocytes, and macrophages from a myelogenous stem cell in studies of patients with chronic myelocytic leukaemia. We have used G-6-PD markers in a study of peripheral blood cells from a patient with acquired idiopathic sideroblastic anaemia, and shown the origin in

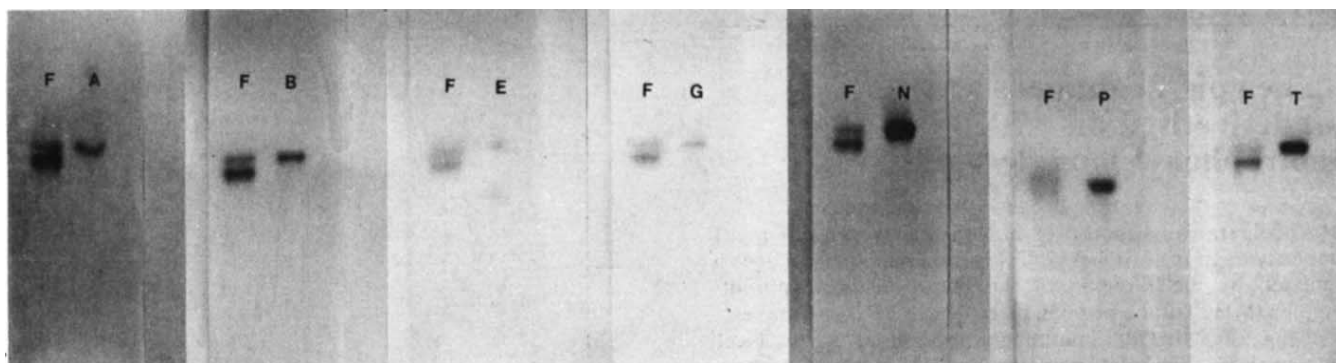


Fig. 1 G-6-PD electrophoresis was carried out on cellulose acetate using the method of Sparkes *et al.*⁹. The tissues were either lysed by probe sonicator in an ice bath or homogenised in a glass homogeniser, frozen and thawed. Membranes were removed by centrifugation. Fibroblasts (F) were cultured in McCoy's 5a media, grown to confluence, trypsinised and collected. Erythrocytes (E) were obtained by filtration through a cellulose fibre column¹⁰. Platelets (P) were obtained by centrifugation at 150g for 10 min at room temperature. Peripheral blood granulocytes, macrophages and lymphocytes were isolated by the Ficoll-Isopaque gradient method¹¹. Granulocytes (G) were obtained from the bottom layer and further purified by sedimentation in Dextran-Isopaque. Granulocytes were 97.5% pure morphologically. Macrophages (A) were obtained from the interphase layer by incubation in Falcon plastic flasks at 37 °C for 1 h. The adherent cells were 79.5% phagocytic with less than 5% granulocyte contamination. Non-adherent lymphocytes (N) were prepared from the interphase layer and separated into T-lymphocyte-rich and B-lymphocyte-rich fractions using the technique of Moretta¹². The T lymphocyte fraction (T) contained 85% SRBC-rosetting cells and 5% fluorescing cells (goat anti-human IgG conjugated with fluorescein isothiocyanate). The B lymphocyte fraction (B) contained 35% fluorescing cells and 30% rosetting cells. The contaminating red cells were removed from all nucleated cell preparations by repeated lysis with ammonium chloride. Although the B lymphocyte purification was not optimal, discriminatory capacity of electrophoretic technique allows detection of less than 5% of a minor band. Thus, it can be concluded that an overwhelming majority of B lymphocytes is G-6-PD-type A. This conclusion is further strengthened by the presence of a denser B over A bands in fibroblasts.

humans of B and T lymphocytes from a common stem cell whose other progeny include erythrocytes, granulocytes, megakaryocytes, and macrophages. In addition, acquired idiopathic sideroblastic anaemia is shown, in this instance, to be a clonal disorder.

The patient is a 67-yr-old black female with previously normal haemoglobin concentrations who was found to be anaemic on routine screening test in January 1977. There was no history of bleeding, dietary deficiency, medications, infections or exposure to known toxic agents. The haemoglobin concentration was 7.8 g% and the reticulocyte count was not increased. Leukocyte count was 7,200 with a normal differential; the platelet count was 300,000. Bone marrow aspiration revealed a hypercellular marrow with a relative increase of erythroid precursors, increased iron stores, and many ringed sideroblasts. Serum iron saturation was increased. Haemoglobin electrophoresis and quantification of HbA₂ and HbF were normal, as were serum folate and B₁₂ levels. Immunoglobulin electrophoresis showed that quantitatively immunoglobulins were within normal limits. There was no response to a therapeutic trial of pyridoxine.

Skin biopsy was carried out to establish a fibroblast culture, and electrophoretic analysis of fibroblast lysates revealed the presence of A and B isoenzymes of G-6-PD. The heterozygosity was confirmed again by electrophoresis of cellular lysates derived from saliva. Peripheral blood cells were separated and analysed for G-6-PD isoenzymes. Spectrophotometric quantitative assay of erythrocyte G-6-PD revealed that the patient has the A⁻ variant. Electrophoretic results are reported in Fig. 1.

The G-6-PD isoenzyme analysis of the erythrocytes, granulocytes, platelets, macrophages, and B and T lymphocytes demonstrated the presence of only one G-6-PD isoenzyme, type A, whereas the skin fibroblasts and salivary epithelial cells contained both isoenzymes A and B. Clonal proliferation of progeny cells has occurred such that mature erythrocytes, granulocytes, platelets, macrophages, and B and T lymphocytes of other clones are either absent or present in insufficient quantity to be apparent on analysis for G-6-PD electrophoretic variants. Thus, acquired idiopathic sideroblastic anaemia in this patient seems to be a clonal disorder. Furthermore, these observations contribute to our understanding of the proliferative capacity of haemopoietic stem cells. Adamson *et al.*¹³ in a study of two patients with polycythaemia vera, have

demonstrated the clonal origin of erythrocytes, granulocytes, and platelets but not of peripheral blood lymphocytes. These observations provided evidence for the existence of a stem cell pluripotent with respect to myeloid cells but without demonstrable progeny in the lymphoid line. Our study documents the existence of a distinct and more primitive pluripotent stem cell capable of differentiating into both myeloid and lymphoid progeny.

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Guinea pig Ia antigens are not derivatised on trinitrophenyl-modified cells

THE proliferative response of hapten-reactive guinea pig T lymphocytes to trinitrophenyl (TNP)-modified macrophages is regulated by the I-region of the major histocompatibility complex (MHC) of the priming macrophage¹. Recent studies^{2,3} have suggested that the immunogen recognised by the T cell contains both TNP determinants and Ia antigens, as proliferation can be specifically blocked by anti-TNP serum as well as by anti-Ia sera directed against the stimulator macrophage. However, the precise nature of the association between the TNP determinant and the Ia antigens forming this immunogen remains unclear. In an effort to clarify the nature of the TNP-specific immunogen recognised by the T cell, we have analysed, by chemical methods, the membrane proteins of TNP-modified guinea pig macrophages. We report here experiments which demonstrate that macrophage Ia antigens are not directly trinitrophenyl-derivatised on TNP-modified macrophages.

Peritoneal exudate cells from strain 13 guinea pigs were derivatised by the method of Shearer⁴ using 10 mM trinitrobenzene sulphonic acid (TNBS), surface labelled with ¹²⁵I by lactoperoxidase catalysis and lysed with detergent. TNP-modified proteins were then precipitated from the cell lysate with anti-TNP serum, and the precipitates were analysed by SDS-polyacrylamide gel electrophoresis. Figure 1 shows that multiple cell surface proteins can be precipitated with anti-TNP serum. In addition to proteins directly bearing the TNP moiety, anti-TNP could potentially precipitate nonderivatised molecules complexed in some fashion to other TNP-bearing proteins. However, the complexity of the electropherogram obtained largely precludes analysis of the manner in which the hapten and Ia antigens might be associated.

To facilitate this analysis, sequential precipitation experiments were carried out. Aliquots of the cell lysate were first precipitated with an excess of normal serum, anti-TNP serum, or anti-Ia serum (prepared as previously described⁵), thereby removing all molecules either bearing or complexed with the antigen recognised by the pretreatment serum. The remaining supernatants were then tested to determine the continued presence or absence of the antigens in question. The results of this experiment demonstrate that all TNP-derivatised molecules in the cell lysate are independent of proteins bearing Ia antigenic determinants (Fig. 2); pretreatment with anti-TNP serum does not demonstrably diminish the Ia antigens precipitable from the lysate, nor does pre-precipitation of the Ia antigens alter the electropherogram observed with anti-TNP serum.

However, if only a small percentage of the cell surface Ia antigens were directly TNP derivatised, it is conceivable that removal of these TNP-Ia antigen molecules would not be detectable in analysing the complex pattern seen with the anti-TNP precipitate. Similarly, minor differences in the amount of precipitable Ia antigen before and after anti-TNP treatment might be missed if the TNP-Ia molecules represented only a small fraction of the total Ia antigen precipitated. To investigate this possibility, cells were internally labelled with ³H-leucine for 6 h, TNP modified and lysed with detergent. In these conditions, the major portion of radiolabelled Ia antigens are membrane associated (unpublished observations) and thus available for TNP derivatisation. The Ia antigens from the lysate were then purified by lentil lectin affinity chromatography followed by anti-Ia immunoabsorbent chromatography and acid elution⁶. Purified Ia antigens were then precipitated with normal serum, anti-TNP serum or anti-Ia serum (Fig. 3). Although Ia antigens were readily detectable by precipitation with anti-Ia serum, no TNP-modified Ia antigens could be

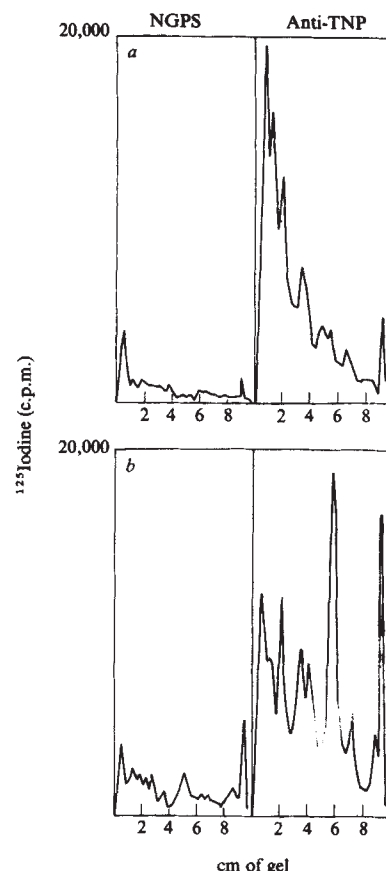


Fig. 1 SDS-polyacrylamide gel electrophoresis of TNP-derivatised membrane protein. Strain 13 peritoneal exudate cells were modified with 10 mM TNBS for 10 min at 37 °C, then surface labelled with ¹²⁵Iodine by lactoperoxidase catalysis and solubilised with 0.5% Nonidet P-40. Following ultracentrifugation, the cell lysate was treated with either normal guinea pig serum (NGPS) or guinea pig anti-TNP serum followed by protein A-bearing *Staphylococcus aureus*. The precipitated complexes were then solubilised in 2% SDS at 100 °C for 1.5 min and analysed on 10% discontinuous SDS-polyacrylamide gels in nonreduced (a) and reduced (b) conditions. Multiple cell surface proteins are specifically precipitated by anti-TNP serum.

demonstrated. In addition, no proteins having the molecular characteristics of Ia antigens could be demonstrated in the unbound effluent from the immunoabsorbent with either anti-TNP serum or anti-Ia serum, making it unlikely that any directly haptenated macrophage Ia antigens failed to bind to the immunoabsorbent as a result of some TNP-induced loss of antigenicity. Finally, whereas acid elution destroys approximately 50% of the Ia antigens eluted from the immunoabsorbent, it does not affect the antigenicity of the TNP determinant because, in parallel studies, TNP-derivatised membrane proteins could be adsorbed to an anti-TNP immunoabsorbent, eluted with acid and reprecipitated with anti-TNP serum. No Ia antigens could be demonstrated in the eluates from these anti-TNP immunoabsorbents (data not shown).

In contrast to our findings, Forman *et al.*⁷ have reported that virtually all H-2K and H-2D antigens on murine splenic lymphocytes are modified by TNBS treatment. This difference cannot be attributed to variations in techniques or reagents, but seems to be a genuine species difference. Studies are in progress to determine the extent to which guinea pig B antigens (analogues of the murine H-2K and H-2D antigens) are derivatised. We can only speculate why cell surface Ia antigens are not derivatised by TNBS treatment. In the conditions used for modification of the cells, TNBS reacts primarily with the

ϵ -amino groups of lysine residues on the cell exterior⁸. Although strain 13 Ia antigens contain lysine (M. Waxdal, personal communication), no information is available regarding the position of these residues with respect to either the tertiary structure of these molecules or their orientation in the cell membrane. Our findings suggest, however, that membrane-associated Ia antigens of strain 13 guinea pigs do not have lysine residues which are readily available for derivatisation by TNBS. This is, perhaps, not surprising, as studies of the reaction of TNBS with intact red blood cells have shown^{9,10} that, depending on reaction conditions, only 0.2–3.4% of the total potentially reactive groups of membrane proteins are derivatised. In addition, Arrotti and Garvin¹¹ have reported that only selected red cell membrane proteins are derivatised on TNBS-treated cells, and Vidal *et al.*¹² have shown that TNBS reacts with only a small number of the total membrane proteins of SV40-transformed fibroblasts, derivatising significantly fewer surface proteins than does lactoperoxidase-catalysed iodination.

Fig. 2 SDS-polyacrylamide gel electrophoretic patterns following sequential precipitations. Identical aliquots of detergent lysates from TNP-modified, radioiodinated cells were initially immunoprecipitated with an excess of the pretreatment serum shown at the left to remove all proteins bearing or associated with the determinant recognised by that antiserum. The remaining supernatant was then treated with the test sera shown at the top of the figure, and the precipitates electrophoretically analysed. Ia antigens and TNP-derivatised proteins are independent of one another in the cell lysate. Although guinea pig Ia antigens consist of two associated protein chains of MW 33,000 and 25,000, the MW 25,000 chain is preferentially iodinated by lactoperoxidase.

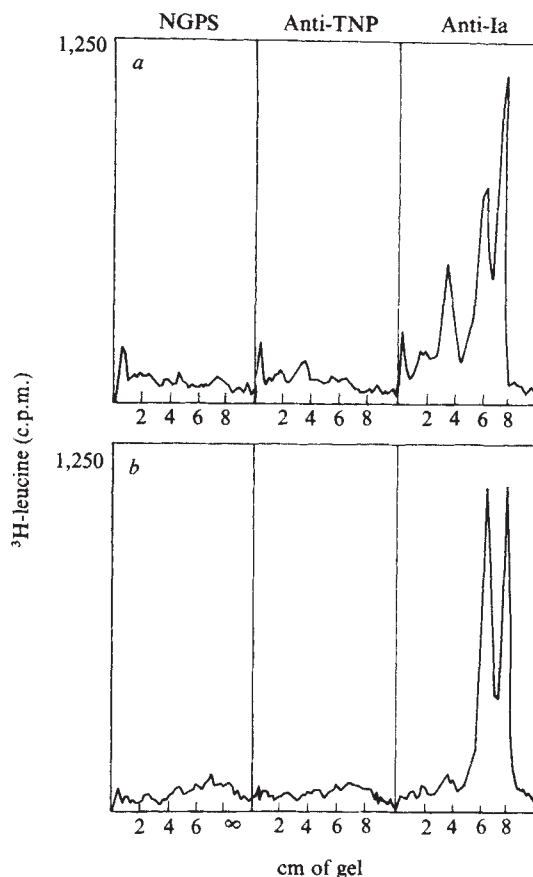
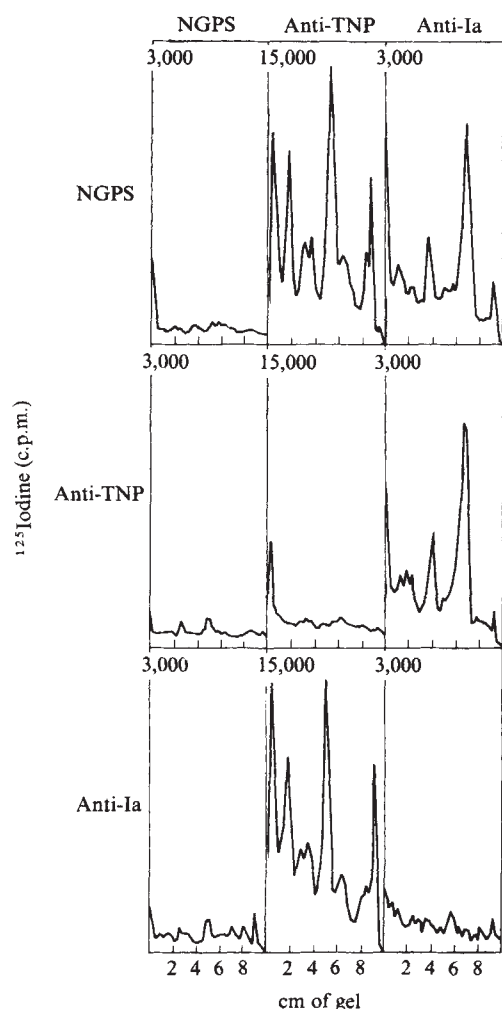


Fig. 3 Electrophoretic analysis of precipitates from purified Ia antigens. Cells were internally labelled with ³H-leucine for 6 h, then TNP-modified and lysed in detergent. A glycoprotein-enriched fraction was then isolated by lentil lectin affinity chromatography, and Ia antigens were purified from this fraction by anti-Ia immunoabsorbent chromatography using 0.2 M glycine HCl, pH 2.8, as the eluant. The purified Ia antigens were then tested with NGPS, anti-TNP or anti-Ia serum in nonreduced (a) or reduced (b) conditions. No Ia antigens are precipitated with anti-TNP serum.

Our experiments thus provide strong evidence that the TNP-specific immunogen which T lymphocytes recognise on guinea pig macrophages does not consist of directly haptenated Ia antigens. Hence, T cells might independently recognise the TNP moiety coupled to membrane proteins and the unmodified Ia antigens by separate receptors. However, Henkart *et al.*¹³ have found that the mere presence of both TNP (in the form of TNP stearoyl dextran noncovalently absorbed to membrane lipid) and H-2 antigens on the cell surface provided for neither the immunogenicity nor the antigenicity of hapten-modified syngeneic stimulator cells in H-2-associated cell-mediated lympholysis. This finding suggests the need for a covalent linkage of the TNP group to membrane protein. The data presented here and elsewhere³ agree with this concept, and although they would seem to exclude the possibility that T cells possess a single receptor which recognises Ia antigens chemically altered by TNP derivatisation, they do not exclude the existence of a single receptor which recognises both TNP and Ia antigens, perhaps in the form of a specific complex on the macrophage membrane. Studies on the nature of this complex and its recognition by T cells are in progress.

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Barbiturate-induced slow outward currents in *Aplysia* neurones

PREVIOUS studies of barbiturates have failed to show significant effects on voltage-dependent membrane conductances at clinically relevant concentrations^{1–3}. As these drugs have effects on synaptic processes at clinical concentrations, some investigators have concluded that the primary site of action of barbiturates is at the synapse^{1–5}. However, we have found that barbiturates at low concentrations (0.05–0.5 mM) can have profound effects on voltage-sensitive conductances in *Aplysia* neurones. Barbiturates suppress the ability of these neurones to fire continuously in response to prolonged depolarisation. A voltage-clamp analysis has revealed the membrane currents underlying this effect, indicating that barbiturates act by enhancement of a slowly developing outward current that appears in response to depolarisation of the cell.

Pleural, pedal, buccal or abdominal ganglia were dissected from *Aplysia californica* (Pacific Bio-Marine) and pinned in a Sylgard-lined chamber. In some cases, the sheath encapsulating the ganglion was dissected to expose the cells. The chamber was perfused with artificial seawater at a pH adjusted to the pK_a of the barbiturate being studied (sodium pentobarbital 8.0 or sodium phenobarbital 7.3). In these conditions, half of the drug exists in the ionised form and half exists in the unionised form. Anterior and medial cells of the pleural ganglion, R₂ cells of the abdominal ganglia and unidentified cells of the buccal and pedal ganglia were studied. Cells were impaled with glass capillary fibre microelectrodes filled with 1.5 M KCl. Once a cell was clamped using the single microelectrode voltage-clamp technique⁶, the potential was held at –60 or –70 mV. Current–voltage (I – V) relationships over a range of –20 mV to –100 mV were studied by giving pulse commands lasting up to 2–5 min. After the control data were recorded and a test flow carried out to insure the stability of the cell, the barbiturate solution was allowed to flow into the chamber.

The action of barbiturates to suppress repetitive firing is illustrated in Fig. 1. In normal seawater, the cell depicted in Fig. 1a was depolarised such that it fired steadily for 100 s. In seawater containing 100 μ M pentobarbital, the cell was again depolarised by the same current. Spike threshold and the initial firing rate were unchanged, but the cell adapted to the stimulus and ceased firing. In these circumstances the cell could be made to resume firing by increasing the depolarising current (not shown), indicating that the adaptation did not result from spike inactivation. However, we did notice that the conductance of the cell was higher in the adapted state than before depolarisation. This, coupled with the fact that the adapted cell remained capable of spiking if further depolarised, led us to consider that the adaptation involved an increase in membrane conductance to an ion which would hyperpolarise the cell. Partridge and Stevens⁷ have proposed that adaptation can result from such a mechanism.

To explore this possibility we used the voltage-clamp technique to determine the I – V relationship of neurones before and after the application of barbiturate. Typical results are shown in Fig. 1b and c. In the control phase the traces ex-

bited an initial outward current which then increased slightly during the course of the command pulse. The two current traces shown on the right for (b) were produced by identical voltage commands 30 min after exposure to pentobarbital 100 μ M. The current began at an amplitude similar to that of the control phase but then slowly increased, as can be seen from the 100-s pulse.

To measure the voltage sensitivity of this slow outward current, the cell was clamped to –60 mV and commanded to potentials from –40 mV to –90 mV. The I – V relationship of the cell was measured at 5 s and 100 s, and then plotted (Fig. 1c). In the control solution there was only a slight difference between the 5-s and 100-s curves, indicating that little outward current develops during the long commands. In pentobarbital 10^{–4} M, the 5-s curve was again minimally changed compared

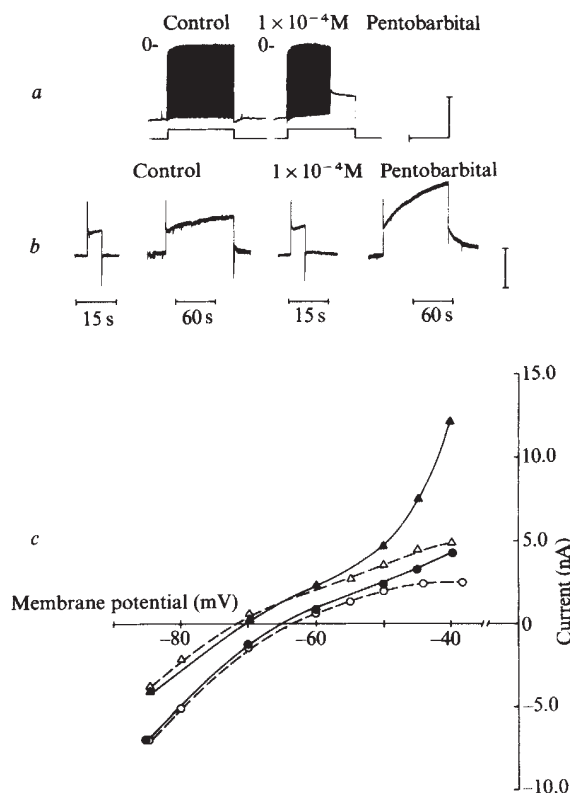


Fig. 1 a, Effects of pentobarbital on an anterior cell of the pleural ganglion. In the control, the cell was set to a potential of –60 mV and then depolarised to fire continuously by a 5 nA current. This was twice the minimum current necessary to elicit spiking in this cell. With pentobarbital 10^{–4} M, the cell was again set to –60 mV and the same depolarising current applied. The cell fired at the same initial rate, but then adapted after 65 s. Further depolarisation of the adapted cell would again elicit spiking (data not shown). Spike threshold (–39 mV) was not altered by the pentobarbital. Action potentials are shown as recorded on a Brush pen recorder. Calibrations, 60 s 30 mV. b and c, voltage-clamp currents from same cell as in (a), derived from depolarising commands from a holding potential of –60 mV to –45 mV for 5 s and 100 s. b, The cell was commanded to –45 mV for 5 s and 100 s in the control phase (left) and pentobarbital-treated phase (right). The barbiturate produced a slow outward current best seen by comparing the 100-s currents. Vertical calibration 7.5 nA. c, I – V plots at 5 and 100 s in the control and with 10^{–4} M pentobarbital for the same cell as in (a) and (b). In the control, the 100-s curve shows a slight outward current between –50 mV and –40 mV compared with the 5-s curve. With pentobarbital the 5-s curve was altered minimally, whereas the 100-s curve showed a dramatic increase in outward current and conductance from –60 mV to –40 mV. Note that pentobarbital also shifted the 5-s and 100-s curves about 1.5 nA outward, indicating a slight hyperpolarisation of the cell. ●, 100 s control; ○, 5 s control; ▲, 100 s pentobarbital 0.1 mM; △, 5 s pentobarbital 0.1 mM.

with the control phase. However, the 100-s curve shows that a strong outward current was clearly present at membrane potentials less negative than -50 mV. The current was highly voltage sensitive. In most cells studied the current was activated at potentials less than -60 mV, and always increased with depolarisation. The effects could be reversed by washing. Both the adaptation effect and the slow outward current enhancement were seen in less than 10 min after drug application. Sequential measurements for extended periods showed that both effects were approximately 50% of maximal in 15 min and complete within 1 h.

It should also be noted (Fig. 1c) that there was a 1.5 nA outward shift in the I - V curves with barbiturate treatment, which correlated with about a 5 mV hyperpolarisation of the cell by pentobarbital. Hyperpolarisations of 1–6 mV were seen with both phenobarbital and pentobarbital, although at the low concentrations used in this study the hyperpolarisations were not consistently seen. Similar small hyperpolarisations have previously been noted by others^{1,2,8}, but the mechanism is not yet clear.

In contrast to the sporadic hyperpolarisation, the enhancement of slow outward current consistently occurred in more than 50 experiments with the groups of identified cells described above. Less than 5% of these identified cells failed to respond to the concentration used in this study. The reason for the lack of effect in these cells is unknown. Both phenobarbital and pentobarbital were found to produce qualitatively similar results, although pentobarbital seemed to be approximately five times more potent than phenobarbital.

We considered that the outward current may have been secondary to a barbiturate-induced release of a neurotransmitter. Experiments were therefore carried out in seawater with a high magnesium concentration to inhibit the release of such substances, but barbiturate enhancement of the outward current persisted in these conditions.

It seems that exposure of *Aplysia* neurones to barbiturates in low concentrations enhances a voltage-sensitive slow outward current which is strongly activated within the firing range of these cells. As the study by Partridge and Stevens⁷ indicated that this current may be mediated by potassium, we tested the sensitivity of the barbiturate-enhanced current to potassium ions.

Figure 2a is representative of the data obtained in ion substitution experiments. The current responses to voltage-clamp commands from -60 mV (holding potential) to -40 mV are shown. In seawater containing a normal concentration of potassium (10 mM) with pentobarbital (100 μ M), there was a considerable increase in slow outward current over control. Seawater containing a low concentration of potassium with barbiturate markedly enhanced the current, whereas seawater containing a high concentration of potassium with barbiturate depressed it.

These data suggest that the slow current is at least partially dependent on potassium ions. We therefore measured the equilibrium potential of the underlying ionic process and its sensitivity to changes in the extracellular potassium ion concentration. Reversal potential of the slow current was determined by studying the 'tail' currents after depolarising commands. The tail current represents the decay of the slow outward current after repolarisation of the cell at the termination of the voltage command pulse. It is best seen in the second and third traces of Fig. 2a (1 and 10 mM $[K^+]_o$). The potential at which the tail current changes to an inward rather than an outward current is the equilibrium (reversal) potential for the ionic process underlying the current.

The reversal potential of the tail current was measured by voltage clamping the cell to a depolarised level (-30 mV) to activate the slow outward current and then commanding the cell to various hyperpolarised potentials. The size and direction (inward or outward) of the tail current was measured at potentials from -30 mV to -120 mV. These experiments were first carried out in control seawater to establish the reversal poten-

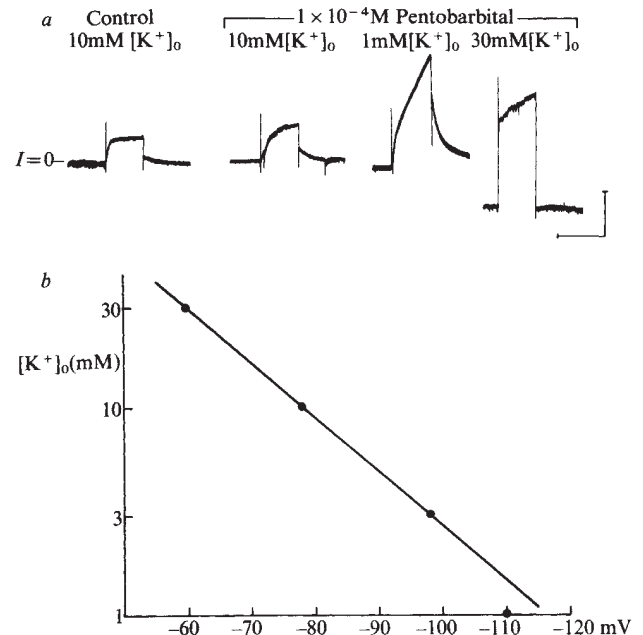


Fig. 2 a, Illustration of the potassium ion dependence of the slow outward current. The left pleural ganglion was clamped to -60 mV then pulsed to -40 mV for 60 s. The current response in normal seawater (with and without barbiturate) and in high (30 mM) and low (1 mM) potassium is shown. It can be seen that pentobarbital 100 μ M enhanced the outward current in normal (10 mM) potassium. The 1 mM concentration further enhanced the current, whereas 30 mM depressed the current. (Note that the high potassium also increased the overall conductance of the cell—a common effect of high potassium unrelated to the presence of barbiturate.) Horizontal calibration 80 s. Vertical calibration 20 nA. b, Amplitude of the tail currents plotted against extracellular potassium concentrations. The slope of the line is 38 mV per decade. Pentobarbital 100 μ M was used.

tial of the slow outward current, and the procedure was then repeated in seawater containing barbiturate at four concentrations of extracellular potassium (1, 3, 10 and 30 mM). The tail current reversal potentials derived from these experiments are plotted in Fig. 2b. In control seawater and in barbiturate seawater with a normal potassium ion concentration (10 mM), the tail current reversed at -77 mV. This is approximately the measured potassium equilibrium potential for these cells⁹. Moreover, at a high extracellular potassium concentration (30 mM) the reversal potential shifted to a less negative value and more negative values were obtained at a lower concentration (1 or 3 mM) of potassium. The slope of the line fitted to these data points was 38 mV per decade change in potassium concentration, indicating that the current was heavily dependent on potassium. The slope, however, was less than the 58 mV per decade potassium ion concentration predicted by the Nernst equation for a current entirely mediated by potassium. It is possible that other ions contribute to the outward current. However, Brodwick and Junge¹⁰ and Partridge and Stevens⁷ reported similar slopes for the potassium current underlying post-stimulus hyperpolarisation and for adaptation, and they could find no evidence of participation by ions other than potassium. Further studies may clarify this issue.

In summary, the data presented here suggest that pentobarbital and phenobarbital enhance a slow, potassium-dependent outward current in *Aplysia* neurones. Previous studies have suggested that such a current could cause adaptation to depolarising stimuli^{7,10–12}. The barbiturates seem to potentiate this process strongly.

Previous reports have described effects of barbiturates on non-synaptic membranes of invertebrates^{2,8,13,14}. For example, burst firing was induced or potentiated in cells prone to this

behaviour^{13,14}, and it was suggested that this might represent an excitatory effect of these drugs. Our series of experiments concentrated on cells other than burst firing neurones and we have not observed excitation of neurones which do not exhibit burst firing. Other studies^{2,8} have shown general depressant effects of barbiturates mediated by increased resting potassium conductance, but this occurred at concentrations in the millimolar range, considerably higher than those found clinically¹⁵. Our data indicate that potent suppression of sustained firing can be seen at lower, more appropriate levels.

Shapavolov and others reported that anaesthetic agents including barbiturates enhanced the adaptation of neurones to depolarising stimuli (refs 16–18, and G. Sourjen, personal communication). The enhanced adaptation impaired the ability of vertebrate neurones to sustain repetitive firing, even though current required to elicit one spike was relatively unaltered. Our observations support these earlier studies and suggest that the development or enhancement of a slow, voltage-sensitive, potassium current could underlie this phenomenon.

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GABA-induced conductance fluctuations in cultured spinal neurones

ANALYSIS of conductance fluctuations induced by putative neurotransmitters has provided a new level of resolution of the molecular events underlying postsynaptic membrane responses^{1–3}. So far, conductance fluctuation (or 'noise') analysis has been applied to neuromuscular synapses in vertebrates and invertebrates^{1–6} and has not yet been applied at synapses in the mammalian central nervous system (CNS), largely because of the inherent complexity and relative inaccessibility of the CNS. Recently, methods for growing dissociated neurones derived from fetal mouse spinal cord in cell culture have become sufficiently established⁷ to allow long-term stable intracellular recording. Using this preparation we have applied fluctuation analysis to the membrane current flow induced by the putative inhibitory amino acid transmitter γ -aminobutyric acid (GABA) and report here an initial estimate of the conductance and duration of ion channels activated by GABA.

Cultures of dissociated spinal cord (SC) neurones were established from 12- to 14-d-old mouse embryo spinal cords

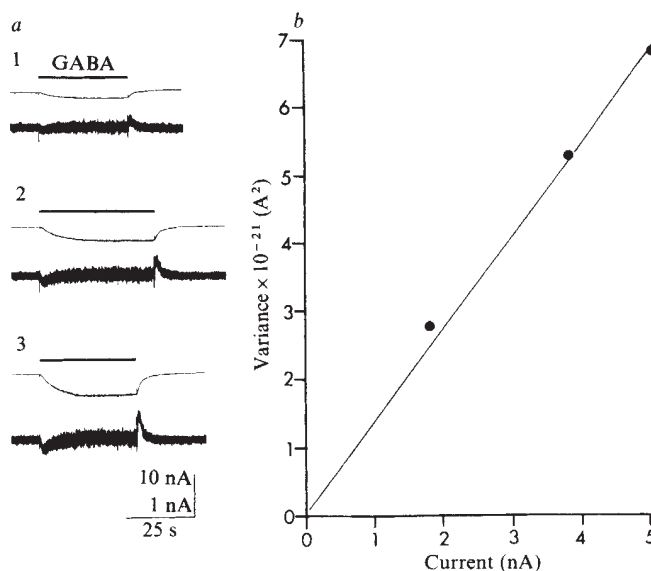


Fig. 1. *a*, Membrane current responses of voltage-clamped spinal chord neurones in cell culture to iontophoretic application of GABA. Steady iontophoretic currents were applied during the times indicated by the black bars above each of the three pairs of records. Each pair contains a d.c.-coupled trace (above) and a high gain a.c.-coupled trace (below). An increase in the thickness or 'noise' of the a.c.-coupled trace accompanied the membrane current response to GABA. The 'noise' increased as the intensity of the iontophoretic current and subsequent membrane current response increases (1–3). Clamp potential (V_c): -80 mV. Temperature: 26°C . *b*, The variance of the noise induced by GABA was linearly related to the change in membrane current. The increase in variance of the noise for each of the three records shown in (*a*) was calculated from digitised records of the noise during the plateau phase of each response and plotted against the corresponding change in membrane current. The points were approximated by a straight line whose slope yields a single channel conductance of 23 pS.

using methods described previously⁷. Individual SC neurones with approximately $20\text{-}\mu\text{m}$ cell bodies bathed in maintenance medium were impaled with two microelectrodes filled with 3 M KCl ($30\text{--}50$ M Ω) while viewed with phase contrast optics at $\times 250$ magnification. GABA was applied by iontophoresis from a third microelectrode positioned close to the cell body. 10 mM MgCl_2 was added to suppress synaptic activity and allow clearer examination of the postsynaptic events. Experiments were carried out at $26 \pm 1^\circ\text{C}$ to retard the onset of desensitisation evoked by prolonged application of GABA (unpublished observations).

In these conditions cells had resting membrane potentials of -30 to -50 mV, input resistances in the range $5\text{--}20$ M Ω , and were electrically excitable when depolarising current was passed through one of the intracellular electrodes. SC neurones impaled with KCl-filled electrodes show inversion potentials of GABA responses which average close to -10 mV (ref. 8). Thus, when recording with KCl electrodes, GABA responses are depolarising at resting potential. Inversion potentials of GABA responses recorded with KAc electrodes average -63 mV (ref. 8). From these observations, and Cl^- replacement experiments, GABA responses evoked at the cell body are thought to be mediated predominantly by an increase in Cl^- conductance⁸. KCl-filled electrodes were chosen for use in these experiments because we found that if KAc electrodes were used it seemed that the Cl^- gradient was significantly altered during the prolonged application of GABA, making fluctuation analysis difficult. Little change in the Cl^- gradient occurred during recordings of GABA-induced response with KCl microelectrodes.

The membrane current flow in response to GABA was assessed by switching the recording system into the 'voltage-

clamp' mode with one microelectrode used to sense membrane potential, V_m , and the other electrode used to pass current to maintain V_m at a command potential, V_c . Figure 1a shows records of membrane current, I_m , in response to the steady iontophoresis of GABA onto an SC neurone the membrane potential of which was clamped at -80 mV. I_m is displayed at two different gains: a low-gain DC-coupled trace (above) and a high-gain AC-coupled trace (below). During the iontophoresis of GABA an increase in I_m coincident with an increase in the baseline thickness or 'noise' of the lower traces can be seen. In all cells studied, and at all potentials, a change in membrane current in response to GABA was always associated with an increase in 'noise'. Although baseline noise depended on the quality of the recording, the average variance was about $1 \times 10^{-21} \text{ A}^2$ (bandwidth: 0.1–1,000 Hz) and the maximum increase in variance produced during the application of GABA was about $2.5 \times 10^{-20} \text{ A}^2$ (bandwidth: 0.1–1,000 Hz), giving a maximum signal-to-noise ratio for variance measurements of about 25:1. The fluctuations were analysed, on the assumption that they derive from the statistical variation in the number of open ion channels activated by GABA around a mean level of open channels^{1–3,9}. If each ion channel is assumed to be of fixed amplitude with a rectangular shape and the probability of any channel being open is small, then an estimate of the amplitude of the single channel conductance, γ , can be obtained from the relationship

$$\gamma = \sigma^2 / (\Delta I \cdot (V_c - E_r)) \quad (1)$$

where σ^2 is the increase in variance above baseline associated with a change in membrane current, ΔI , produced by GABA, and E_r is the reversal potential for the GABA response current.

In Fig. 1b increases in variance during the plateau phases of three responses to GABA (Fig. 1a) are plotted against their respective mean current changes. The points closely approximate a straight line which yields a single-channel conductance of 23 pS. Estimates of the single-channel conductance made in any one cell at different clamp potentials in the range -10 to -90 mV yielded similar results. For example, in one cell the values (mean $\pm$ s.d.) for γ were (pS): 16.2 (-30 mV, $n = 3$), 19.4 $\pm$ 3.1 (-50 mV, $n = 12$), 18.5 $\pm$ 4.2 (-70 mV, $n = 12$) and 21.9 $\pm$ 4.1 (-90 mV, $n = 7$). The overall mean for 271 estimations of the conductance of a single channel activated by GABA for 12 SC neurones was 18.0 $\pm$ 8.2 pS (mean $\pm$ s.d.).

Further information about the characteristics of these ion channels was obtained by carrying out spectral analysis on the fluctuations during the plateau phase of the current responses. Figure 2 is a power density spectrum of the fluctuations in membrane current induced by GABA. Power density spectra were calculated as the Fourier Transform^{10,11} of 4096, 8192 or 16384 point samples of the noise digitised at 1 or 2 ms per point. Spectra of GABA-induced noise were generally approximated by a single Lorentzian^{10,11} of the form $S(f)/S(0) = 1/[1 + (f/f_c)^2]$, where $S(f)$ is the spectral density as a function of frequency, f , and f_c is the frequency where the spectral density has dropped to half the value of the zero-frequency asymptote, $S(0)$, of the spectrum. This is consistent with the hypothesis that the fluctuations arise from a single population of channels the duration of which is exponentially distributed^{1–3,9}. The average duration, τ , of the channels was calculated from the equation $\tau = 1/2\pi f_c$. For 84 estimations of τ in four cells the value was found to be 20 $\pm$ 6.6 ms (mean $\pm$ s.d.). In these experiments we found little dependence of the average channel duration on membrane potential over a 50 mV range.

These results for single channel conductance and duration can be compared with only one result for an anion-specific channel. Dudel *et al.*¹² have reported that GABA-induced conductance fluctuations can be seen in crayfish muscle and that the 'fast' channels have a conductance of about 14 pS and a duration of about 1.2 ms. Our single-channel conductance estimates compare favourably with those reported by Dudel *et*

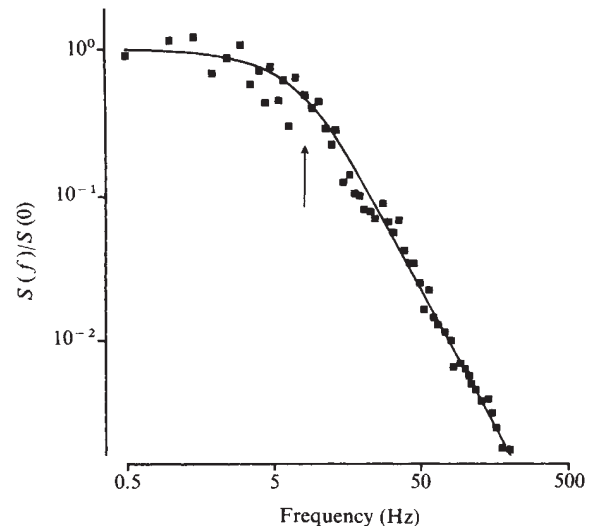


Fig. 2 Power spectrum of GABA-induced current noise recorded from a voltage-clamped spinal chord neurone. The spectrum, obtained as the difference between a spectrum calculated from current noise during application of GABA and a spectrum of baseline current noise, has been normalised by dividing each spectral density point $S(f)$ by the zero-frequency asymptote of the spectrum $S(0)$. Above 10 Hz averaging of spectral points across frequency bands has been carried out to smooth the spectrum and reduce the number of plotted points. The spectral density points were approximated by a Lorentzian function (solid line) described by the equation $S(f)/S(0) = 1/[1 + (f/f_c)^2]$, the half-power frequency (at arrow), $f_c = 7.5$ Hz, of which corresponds to an average channel lifetime of 21.2 ms. Clamp potential, $V_c = -70$ mV. Temperature: 27 °C.

*al.*¹², but there is little agreement between channel lifetimes. There is no reason to assume that there should be any similarity between GABA receptor-coupled anion-specific channels in invertebrate muscle membrane and vertebrate CNS neurones. It is interesting to note, however, that to date most chemically activated ion channels (cation- or anion-specific) have been found to have a conductance in the range 10–50 pS.

We have recently begun to investigate the properties of channels activated by other putative inhibitory and excitatory transmitter candidates and to study the effects of clinically important drugs which alter the postsynaptic responses to these amino acids. These studies, coupled with investigations of naturally occurring synaptic events should provide new insights into the physiology and pharmacology of receptors mediating synaptic events in the CNS and the mechanisms of action of CNS-active drugs at the single channel level.

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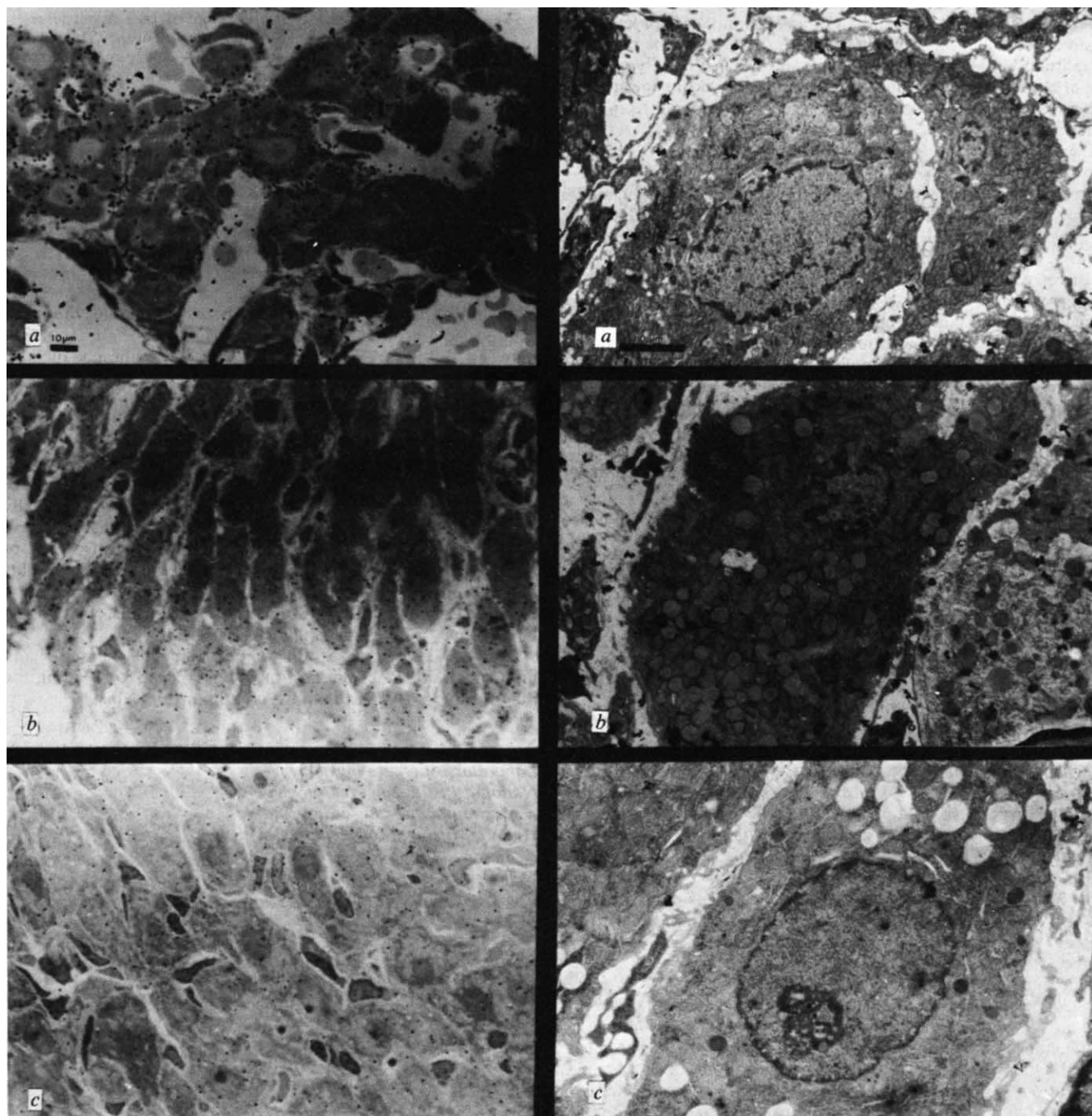
Internalisation of gonadotrophin-receptor complex in ovarian luteal cells

It is generally accepted that peptide and protein hormones bind to plasma membrane receptors on target cells¹ and elicit their acute responses without entering the cell. The refractory or desensitised state induced by various hormones in target tissues has been shown to be mediated at least in part by loss of specific receptor sites from the plasma membrane²⁻⁷. Evidence that certain protein hormones can enter target cells⁸⁻¹⁰ led us to

the present investigation which provides evidence that gonadotrophin-induced receptor loss may occur by a process of internalisation of the hormone-receptor complex following the initial interaction of gonadotrophin with the cell surface.

Localisation studies were carried out in 33-d old female rats previously treated with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) to induce ovarian luteinisation. Animals were injected in the jugular vein with 2×10^6 d.p.m. (100 ng) of ^{125}I -hCG to label the ovarian receptors for luteinising hormone (LH) *in vivo*. In some experiments, $2\mu\text{g}$ of unlabelled hCG was given by subcutaneous injection 30 min later to ensure maximum receptor occupancy; this treatment did not alter the subsequent pattern of translocation of radioactivity. Following the injection of

Fig. 1 Left, light microscope autoradiographs showing distribution of ^{125}I -hCG in ovaries removed 3 h (a), 7 h (b) and 24 h (c) after a desensitising injection of hCG. Right, electron micrographs showing autoradiographic localisation of grains from ^{125}I -hCG in ovarian cells 3 h (a), 7 h (b) and 24 h (c) following injection of tracer hormone. Bar = $10\mu\text{m}$.



labelled hCG, rats were killed at 3, 7 and 24 h, and the ovaries removed into ice-cold fixative. After standard dehydration and embedding procedures, the ovaries were sectioned and prepared for light and electron microscopic autoradiography. Unfixed tissue from identically treated animals was frozen on dry ice and stored at -60°C before density gradient analysis and gel electrophoresis.

The ovarian levels of ^{125}I -hCG at 3, 7 and 24 h after injection were 61.4 ± 3.5 (s.e.), 52.5 ± 7.5 and $3.7 \pm 0.5\%$ ($n=3$) respectively, of the administered dose of hormone. The distribution of ^{125}I -hCG at these times is shown in Fig. 1, left, and the quantitative data are presented in Table 1. In all sections examined at 3 h, there was prominent localisation of radioactivity at the plasma membrane, corresponding to 57% of the grains counted. The location of the bound radioactive hormone at 3, 7 and 24 h is seen at higher resolution in the electron microscope autoradiographs shown in Fig. 1, right. Although the grains were located predominantly at the level of the plasma membrane at 3 h, the luteal cells showed considerable internalisation of radioactivity at 7 h, corresponding to the quantitative changes detected by light microscopy. When the majority of the bound radioactivity had been cleared from the ovary, and corresponding to the time of maximum receptor loss at 24 h (refs 4, 13), cells contained only a few grains of radioactivity and these were rarely (3.2%) over the plasma membrane.

To demonstrate the saturable nature of the intracellular translocation of radioactivity, and to exclude nonspecific uptake mechanisms such as non-receptor mediated pinocytosis, a 1,000-fold excess of unlabelled hormone was injected simultaneously with the ^{125}I -hCG in certain experiments. In these conditions, very little radioactivity was detectable in the autoradiographs at any time. The tissue specificity of the uptake and internalisation of ^{125}I -hCG was indicated by the finding that very few grains were associated with cells other than luteal cells, such as erythrocytes and fibroblasts. To determine whether the intracellular radioactivity was due to a fragment of the hormone molecule, the particulate radioactivity was analysed by electrophoresis in 15% polyacrylamide gel following homogenisation of the frozen ovaries in hot SDS and β -mercaptoethanol. In these conditions, essentially all of the tissue radioactivity migrated with the α -subunit of hCG (Fig. 2). On electrophoresis in the same denaturing conditions, the radioactivity from ^{125}I -hCG tracer also migrated with the α -subunit, as previously reported¹⁴.

The subcellular distribution of radioactivity after homogenisation in phosphate-buffered saline showed that more than 75% of the ^{125}I -hCG was localised in the 27,000g pellet at all times examined. This suggested that the radioactivity was associated with macromolecular structures such as membrane fragments or organelles. After solubilisation of the 27,000g pellet with Triton X-100 and analysis by density gradient centrifugation as previously described¹⁵, the bound radioactivity at each time migrated as an 8.8S species (Fig. 3), identical with the hormone-receptor complex formed *in vivo* and *in*

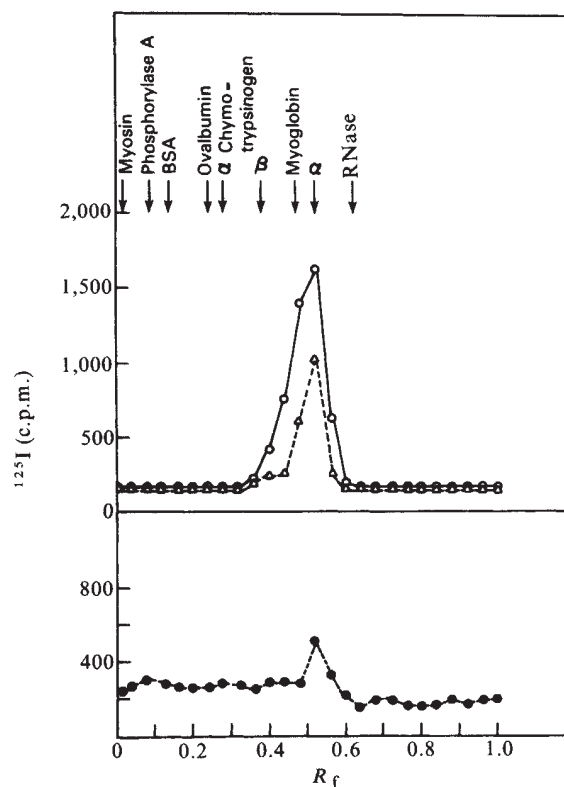


Fig. 2 Polyacrylamide-SDS gel electrophoresis²⁷ of ovarian homogenates prepared 3 (○), 7 (△) and 24 (●) h after administration of ^{125}I -hCG. Migration of protein standards and the α and β subunits of hCG are shown.

*vitro*¹⁶. In contrast, free ^{125}I -hCG sedimented as a 2.9S peak of radioactivity in these conditions¹⁷.

In this study, we have combined autoradiography and solubilisation to determine the location and nature of the hCG-receptor complex following occupancy and loss of receptors from the plasma membrane of luteinised ovarian cells. The data have indicated that the hCG-receptor complex is formed initially at the plasma membrane, then enters ovarian cells in conditions that are accompanied by extensive loss of receptors from the plasma membrane². These findings are consistent with an earlier report that ^{125}I -hCG is predominantly located at the plasma membranes of rat luteal cells when examined 60 min after hormone injection, before the onset of receptor regulation and intracellular localisation¹⁸. Our studies show that localisation was still prominent at the cell membrane after 3 h, as recently demonstrated in ovine luteal cells¹⁹. After longer periods, internalisation of the hormone-receptor complex became more extensive and most of the grains were within the cells by 7 h.

Our observations that the gonadotrophin-receptor complex is located inside target cells following hormone-induced receptor loss⁴ suggests that this process may be mediated through cellular uptake of receptor sites, perhaps still associated with components of the plasma membrane. The entry of proteins and other macromolecules into cells is now a well-recognised process, and is apparently a common consequence of ligand binding. Of particular relevance to our results are observations that cells containing receptors for lipoproteins²⁰, lectins²¹, immunoglobulins²⁰ and hormones^{8-12,19,22-25} may permit these proteins to enter the cytoplasm. Also, radioactivity from labelled gonadotrophins has been shown to enter gonadal target cells and to become compartmentalised in lysosomes, suggesting that hormone metabolism depends on lysosomal acid hydrolases^{19,25}. The intact hormone-receptor complexes observed during internalisation in the present study presum-

Table 1 Distribution of grains in ovarian sections analysed by quantitative morphometry after autoradiography

Hours following injection	3	7	24
Cell profiles counted	6,000	6,000	6,000
Grains counted	151,028	72,608	19,222
Grains per profile (mean)	25.2	12.1	3.2
Grains within 2 HD of the plasma membrane (% of total)	86,276 (57.1)	8,811 (12.1)	608 (3.2)

Quantitative light morphometry²⁶ was carried out on grains using half distance (HD) determinations²⁶ made four our emulsion conditions. The HD is the distance from a developed grain with a 50% probability of containing the source. Grains were classified based on the perpendicular distance to the plasma membrane.

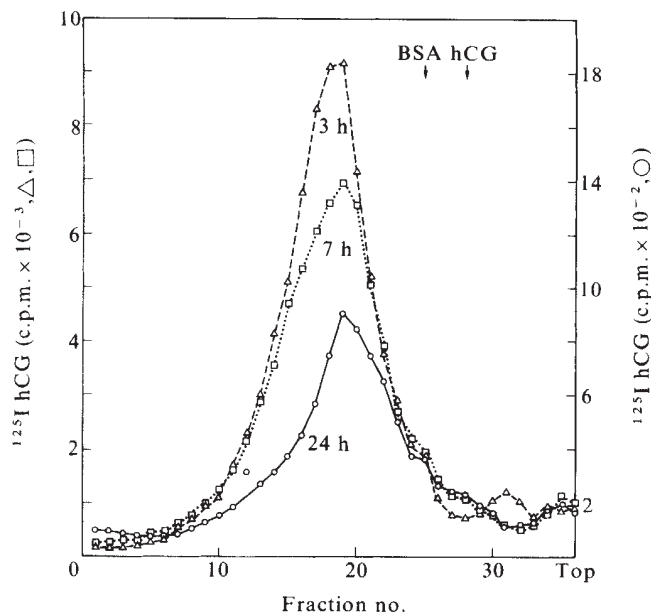


Fig. 3 Density gradient centrifugation of Triton X-100-solubilised ovaries labelled *in vivo* with ^{125}I -hCG. When the solubilised material was analysed in 5–20% sucrose gradients, the 8.8S peaks of radioactivity were precipitable by polyethylene glycol, consistent with the behaviour of the detergent-solubilised hormone–receptor complex of the luteinised rat ovary¹⁵. The left ordinate for the 3- and 7-h time points is different from the right ordinate for the 24-h time point.

ably pass to the lysosomes and undergo catabolism to iodinated degradation products that are rapidly cleared from the cell.

The finding that the hormone–receptor complex enters target cells raises the possibility that certain trophic responses could be mediated through the intracellular hormone. In this regard, it is interesting that no systematic localisation of grains appeared over the nuclear compartment, suggesting that there is no direct influence of gonadotrophin on the nuclear transcriptional systems. Our results suggest that intracellular translocation of the hormone–receptor complex occurs concomitantly with loss of plasma membrane receptors, but we do not exclude the existence of other mechanisms of hormone-induced receptor loss (such as receptor inactivation, sequestration within the plasma membrane or release into the extracellular space). It is also evident that changes in membrane turnover may have a role in gonadotrophin receptor loss. However, the parallelism between the internalisation of the hCG–receptor complex and its loss from the plasma membrane suggests that the former is involved in the process of hormone-induced receptor regulation.

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Removal of whiskers in young rats causes functional changes in cerebral cortex

SENSORY deprivation or deafferentation has been shown to result in a variety of changes within the mammalian central nervous system (CNS)^{1,2}. Although the majority of the studies have been anatomical in nature, recordings from cells in the visual cortex have shown that functional modifications also occur as a consequence of a deprived or abnormal visual experience in early life^{3–6}. Moreover, alterations in neural responses are found in the somatosensory system^{7–9} following removal of input from the hind leg. We report here functional changes in the rat cortex resulting from early destruction of the whiskers. The region of the rat cortex receiving the sensory input from the whiskers contains aggregations of cells known as barrels and these aggregations do not develop if the corresponding whiskers are destroyed soon after birth^{10–13}. We have found that such whisker removal also causes the associated cortical cells to become functionally reconnected with regions of the face surrounding the whiskers.

The whiskers were destroyed unilaterally during postnatal day 1 (a current of 200–400 μA for 30 s passed through a needle inserted into 15 sites on the whisker pad). This usually resulted in the permanent removal of all or most of the whiskers on one side of the face, but occasionally a few stunted whiskers regrew. After 15–26 weeks the animals were anaesthetised with Nembutal (60 mg per kg, then as required), a bilateral craniotomy carried out and microelectrode recordings made at 0.5-mm intervals over the whole of the face region of both cortices. Gentle mechanical stimulation was used to study receptive fields at each cortical site. Recording sites within the face region were marked with dye for later histological measurements.

A total of 12 normal and 9 whisker-deprived cortices were mapped. Recordings were made from both single cells and clusters of cells in all layers except layer I. The map from the normal cortex (Fig. 1a) was similar in the different animals in overall organisation, total area and absolute position and agreed with that reported previously^{11,14,15}. Receptive fields varied over 1–10 whiskers, one cell commonly being strongly driven from one (or occasionally two) whiskers and weakly driven from most of the surrounding whiskers (average seven, Fig. 1a). Great care was taken to ensure that these large fields were not artefacts resulting from whisker movements spreading to adjacent follicles. Thus, receptive fields would seem to be

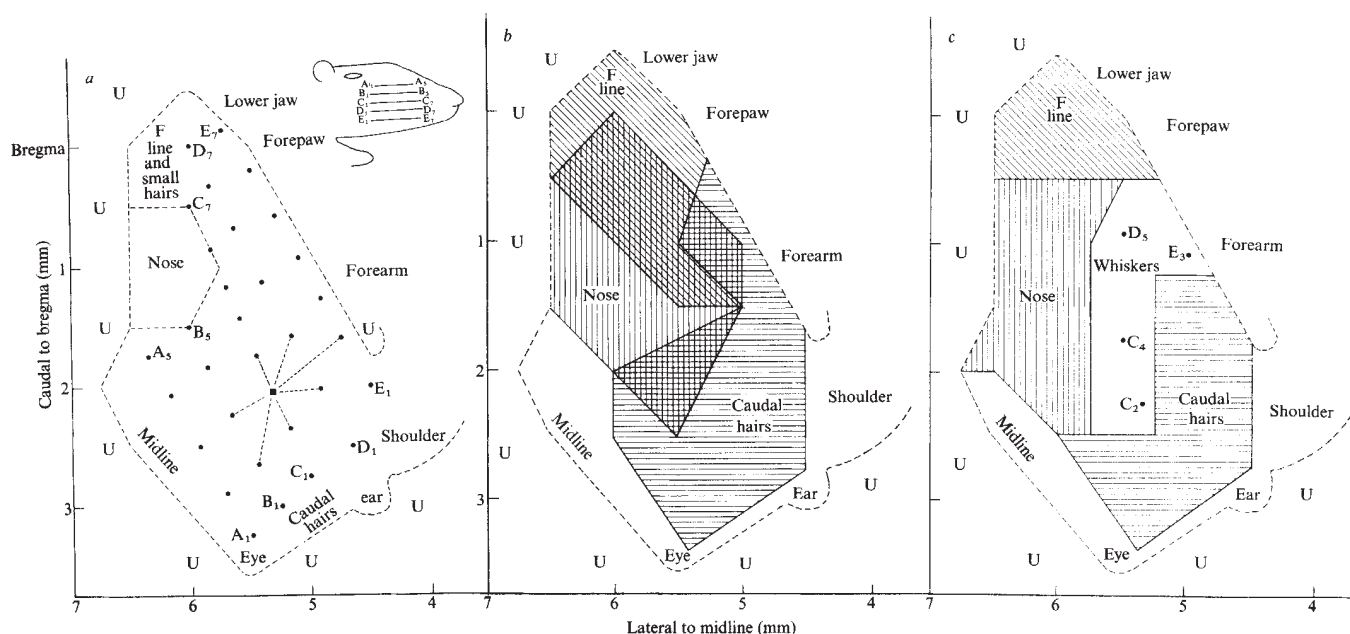


Fig. 1 Organisation of the face region of the rat somatosensory cortex. *a*, The map obtained from the normal cortex. The nomenclature used for referring to individual whiskers is shown inset. In the cortex the whiskers occupy approximately half of the total face region and are organised in rows as in the periphery. Each dot (•) marks the centre of the cortical region for the corresponding whisker. All the whiskers indicated by the radiating dashed lines represent a typical peripheral receptive field for cells recorded from a track maximally sensitive to whisker C₃ (■). *b*, Map obtained from cortex contralateral to the completely whisker-lesioned periphery. All regions normally responsive to the whiskers now respond to stimulation of the contralateral nose, cheek hairs and F line hairs. *c*, Map obtained from cortex contralateral to a partially whisker-lesioned periphery (whiskers C₂, C₄, D₅, E₃ present). These whiskers gave responses in the cortex at their normal sites but over considerably reduced areas. However, the presence of these whisker responses limited the expansion of the nose, F line and cheek hairs. U indicates sites at which cells could not be driven by stimulation of the periphery.

larger than those found by Welker¹¹ and agree more closely with the overlapping projections described by Axelrad *et al.*¹⁵.

The map obtained from the cortex contralateral to the completely lesioned whiskers is shown in Fig. 1*b*. The total face area was of the same size (Table 1) and was recorded in the same position as in the normal cortex. However, cells in the part of the cortex normally responding to whiskers now responded to the surrounding areas of the face, namely the nose, F line hairs (line of small hairs along the lip) and cheek hairs (small hairs lying on the cheek behind the whiskers). The cortical areas for these structures increased to the extent that all parts of the face region of the cortex were responsive and no silent, non-drivable regions remained (typical values are shown in Table 1). Although different animals showed small variations in the shape of the enlarged areas, the areas responding to stimulation of the nose and F line hairs always increased medially and caudally, whereas the area for the cheek hairs expanded rostrally.

Although not defined in detail, cells in the newly connected regions of the cortex seemed to have similar receptive fields, synaptic connections and latencies to those of normal cortex. For example, receptive fields within the normal nose region of the cortex were restricted to small areas on the nose, usually less than 2 mm². Similar receptive fields were also found in the expanded nose representations. In addition, it was the part of the face directly bordering the whiskers which was involved in the expansion into the cortical whisker region. Thus, rostral

nose expanded into rostral whisker regions, whereas the side of the nose expanded into more caudal whisker regions.

In four animals, 1–6 stunted whiskers were found on the lesioned whisker pad. Responses from these whiskers were recorded at their normal positions on the cortex (Fig. 1*c*). The area associated with any such whisker was never larger than normal and was often greatly reduced, probably reflecting the reduced innervation of these stunted structures. However, the presence of such whisker representation prevented surrounding regions of the face from taking over the whole whisker area.

One explanation for the changes in cortical representation reported here is that whisker lesioning may be followed by the regeneration of peripheral nerve fibres to innervate the surrounding skin regions. However, Cragg and Waite¹⁶ showed that similar lesions in mice resulted in degeneration of many of the peripheral fibres. In our study, the total number of myelinated nerve fibres in the maxillary nerve of two lesioned animals (counted at four months of age) was 6,908 and 7,162 (mean 7,035), compared with values of 18,000 and 15,354 (mean 16,677) on the normal sides. Moreover, maxillary nerve branches supplying the nose and F line hairs in a normal animal contained 1,135 and 4,302 myelinated fibres, respectively. Thus, the normal innervation of the nose and F line hairs would alone account for most of the nerve fibres on the lesioned side. In addition, the lesioned nerve also supplies the cheek hairs and skin of the midline and upper lip and thus there is no evidence for an increased innervation of any peripheral regions. The fact that receptive fields of cells are similar in both the normal and expanded cortical representation suggests that there has been little change in terminal ramification of fibres in the periphery.

Possible mechanisms which could result in the present functional changes within the CNS are the unmasking of pre-existing but previously ineffective connections, or the sprouting or regrowth of new connections¹⁷. If the unmasking of ineffective synapses occurred then functional changes should be apparent in the acute preparation^{8,18}. This was tested in three animals by mapping the normal cortex before and after sectioning all or part of the nerve supply to the whiskers. For

Table 1 Cortical areas responding to various regions of the periphery in a unilaterally whisker-lesioned rat

	Total face	Whiskers	Nose	F line hairs	Cheek hairs
Normal side	8.8	4.8 (55%)	1.2 (14%)	0.7 (8%)	0.4 (5%)
Lesioned side	8.9	0 (0%)	3.6 (40%)	2.1 (24%)	1.9 (21%)

Areas are expressed in mm², with % of total face area in brackets. Values are calculated from histological measurements allowing for curvature of the cortex.

four hours after total sectioning no increase in the extent of the nose, F line hairs or cheek hairs could be detected, whereas after partial cuts the area of cortex responding to the remaining innervated whiskers was normal. Thus, no evidence for the unmasking of ineffective synapses could be demonstrated either within the whisker region or between whiskers and surrounding regions.

The occurrence of sprouting within the mammalian CNS is now well established¹⁹⁻²² and we would suggest that this is the most likely explanation for the present findings. The stimulus required to initiate sprouting is unclear although both degeneration products and trophic factors have been implicated^{9,23}. In addition, in the visual system normal electrical activity is important²⁴, and whisker removal has been shown to deprive cortical neurones of normal activity²⁵. The site of the suggested sprouting could be the trigeminal nucleus, thalamus or cortex. There is evidence for the appearance of new synapses on deafferented cells of the trigeminal nucleus²⁶. Indications from preliminary experiments in progress in our laboratory suggest that the present cortical changes may well result from sprouting of intact primary afferents ending in the trigeminal nucleus.

In several studies, the plasticity of the nervous system has been found to decrease with age^{24,27,28}. It was therefore of interest to test the consequences of lesions in adult animals. However, whisker lesioning in adult animals was found to result in regeneration of the peripheral nerve fibres to the surrounding skin. This peripheral regeneration has complicated the interpretation of any central changes and thus it has not been possible as yet to provide evidence of any critical period for the reorganisation of the whisker pathway, comparable to that in the visual system^{3,6,29}.

It is interesting that receptive fields of cells in the newly connected cortical regions were similar to those of normal cortex. In similar studies in the dorsal horn⁴ and dorsal column nuclei⁵ evidence for double fields, large inhibitory fields and habituation was given. This discrepancy may reflect differences between the trigeminal and dorsal column path or could be the result of recording at cortical level compared with earlier stages along the afferent pathway. Finally, we cannot say to what extent the increased areas of cortex may reflect an increased sensitivity or discriminative ability of the associated peripheral regions. However, it is possible that the present functional changes may be behaviourally advantageous, and hence form part of a useful neural recovery mechanism.

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Organic lead in normal human brains

LEAD-CONTAINING organic compounds such as tetraethyl and tetramethyl lead have been used for over 50 years as anti-knock additives to petrol. The occupational hazards associated with their production and use are well documented, but no adverse effects of environmental pollution by organolead compounds have yet been demonstrated. Here we present an analysis of 22 human brains which shows that a significant part of the lead content may be organolead. The highest concentrations (median, 2×10^{-7} mol per kg) were present in individuals residing in the lower floors of buildings in the city.

Most of the tetraalkyl lead in petrol is decomposed to inorganic lead during the combustion in the automobile engine. Evaporation and incomplete combustion of leaded petrol, however, cause atmospheric pollution by organolead compounds. The 24 h mean value of volatile tetraalkyl lead is about $0.25 \mu\text{g m}^{-3}$, and it constitutes about 10% of the total airborne lead in urban streets^{1,2}. Moreover, about 1% of the lead in particles is probably organic.³ Tetraalkyl lead is thought to be quickly photolysed in the atmosphere^{4,5}. However, a very small part of the solar UV radiation at the surface of the earth is below 300 nm (ref. 6), and tetramethyl and tetraethyl lead vapours are not photolysed above 280 nm (refs 7, 8). Thus, the breakdown of these compounds may be much slower than previously thought. Tetraalkyl lead is readily absorbed through the skin and the respiratory tract⁹ and metabolised into trialkyl lead compounds responsible for the toxic effects^{10,11}. The brain is the critical organ in cases of lead poisoning.

Brains from 14 males and 8 females (ages 12-54 yr), residents of Copenhagen, its suburbs and of villages on Zealand, were collected at autopsy. The brains were homogenised and analysed for trialkyl lead, total lead, copper and potassium. The methods of analysis for total lead, copper and potassium are described elsewhere¹². Added trimethyl lead chloride was recovered by $48 \pm 12\%$ and triethyl lead chloride by $67 \pm 11\%$, both as inorganic lead, at the analysis of total lead.

Trialkyl lead compounds were extracted by a modification of Hayakawa's method¹⁰: 1 g of homogenised brain was suspended in 0.4 M nitric acid. After cleaning the water phase with benzene, sodium chloride was added, and the trialkyl lead chlorides were extracted with ethyl acetate and determined directly by atomic absorption spectroscopy at 283 nm with deuterium background correction. No detectable absorption occurred at 287 nm, indicating minimal interference from smoke. Recovery of added trimethyl lead chloride was $101 \pm 9\%$, and of triethyl lead chloride $68 \pm 7\%$. The mean coefficient of variation was 11% for organic lead concentrations exceeding $0.02 \mu\text{g per g}$ wet weight and 39% for lower levels. No extractable lead was found in an archaeological sample of mummified brain which contained as much inorganic lead as contemporary brains¹². Moreover, addition of $1 \mu\text{g}$ lead nitrate to the brain samples did not change the results. Thus the extractable lead is probably trialkyl lead chloride and the presence of inorganic lead does not interfere with the analysis.

Individuals residing in lower floors of buildings in Copenhagen had a significantly higher content of 'trialkyl lead' than individuals from higher floors, or from suburbs and villages

Table 1 Concentrations of 'trialkyl lead', total lead, and copper in brains from 22 individuals

Residence	N	R ₃ Pb ⁺		Total Pb		Cu ²⁺	
		Median	Range	Median	Range	Median	Range
Copenhagen							
lower floors	8	0.039	0.012–0.050	0.067	0.051–0.152	3.23	2.13–4.60
upper floors	4	0.011	<0.010–0.018	0.087	0.059–0.112	3.51	3.26–4.09
Suburbs and villages	10	0.011	<0.008–0.026	0.075	0.044–0.183	3.50	2.55–4.20

'Trialkyl lead' (R₃Pb⁺) was determined as Pb from the calibration curve of trimethyl lead chloride. Values are given as µg lead per g wet weight.

(Kruskal–Wallis test: $P < 0.05$) (see Table 1). These results reflect a much larger difference in the air pollution because an individual's residence is an incomplete indicator of his exposure.

The content of 'trialkyl lead' ranged from less than 4% to 84% that of total lead, and the two concentrations were not correlated. This is in accordance with the fact that many sources other than combustion of leaded petrol contribute to the human burden of inorganic lead. The content of 'trialkyl lead' decreased with increasing age of the individual (Spearman's $r_s = -0.46$; $P < 0.05$), but there was no sex difference (Mann–Whitney U -test; $P \sim 0.35$). A negative correlation between the concentrations of 'trialkyl lead' and copper was found ($r_s = -0.42$; $P \sim 0.05$). The brains with the highest amount of 'organic lead' contained, in mean, 20% less copper than the other brains. These differences in copper concentrations were not related to variations in the content of dry matter or age of the individuals, and there was no correlation between the content of 'trialkyl lead' and potassium ($r_s = -0.13$; $P > 0.5$). It may be relevant that marked changes in copper levels in rabbit brain tissue have been found after lethal tetraethyl lead poisoning^{13,14}. Thus, the possible interference of organic lead with copper metabolism needs further study.

In brains of individuals dying from acute poisoning with tetraethyl lead, about 10 µg lead per g wet weight has been found^{11,15}. Thus the 'organic lead' content in the background population studied ranges from less than 0.1% to about 0.5% of the lethal concentration. It is not possible yet to evaluate the health risks associated with long-term low-level exposure to organic lead compounds. *In vitro* experiments have shown, however, that 10^{-7} mol l⁻¹ (0.02 µg Pb ml⁻¹) triethyl lead chloride inhibits enzymes, for example glutathione-S-aryl-transferase¹⁶ and serum choline esterase¹⁷. Trialkyl lead compounds induce an increased anion permeability of cell membranes¹⁸, and this effect is probably the basic molecular mechanism for the uncoupling of the oxidative phosphorylation occurring at triethyl lead concentrations exceeding 10^{-7} mol l⁻¹ (refs. 18, 19). In view of the widespread pollution by organic lead compounds, evaluation of the effects of their accumulation in human tissues is desirable.

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Hypersensitivity to noradrenaline in cortex after chronic morphine: relevance to tolerance and dependence

OPIOID agents affect the activity of noradrenergic neurones in brain in the following ways: their firing rate recorded in the locus coeruleus is depressed^{1,2}, noradrenaline (NA) levels or turnover is altered³ and the depolarisation-induced release of NA from cerebral slices is inhibited^{4,5}. This latter effect suggests that opiate receptors responsible for an inhibition of noradrenergic transmission might be localised on noradrenergic neurones not only on the cell bodies⁶ but also on the nerve terminals. Because a sustained decrease in noradrenergic transmission in brain results in the development of hypersensitivity of target cells to NA^{7,8}, it was of interest to assess the involvement of such a process in the long-term effects of opiates. We report here that opiate receptors are presynaptically localised on noradrenergic neurones as indicated by their depletion in cortex and cerebellum after a selective degeneration of these neurones, and that chronic treatment with morphine is followed by an increased responsiveness of the cortical cyclic AMP system to catecholamines, accompanied by an increased number of β -adrenergic receptors.

The presence of opiate receptors on noradrenergic nerve terminals was investigated by measuring ³H-naloxone binding in brain regions receiving noradrenergic inputs, after chemical destruction of noradrenergic cell bodies. For 15–40 d after the slow unilateral infusion of 6-hydroxydopamine (6-OHDA) into the locus coeruleus, placement of the lesions was checked histologically and their efficacy assessed biochemically by evaluating the extent of the decrease in ³H-NA uptake in various areas (Table 1). ³H-NA uptake was reduced by about 60% in most regions; the absence of change in the striatum is probably due to ³H-NA uptake into dopaminergic neurones not affected by the lesion, whereas the somewhat higher decrease in cerebellum might be attributed to a diffusion of 6-OHDA into a region close to the injection site. These lesions resulted in a significant decrease in ³H-naloxone binding in

Table 1 Effect of 6-hydroxydopamine-induced lesion of locus coeruleus on ^3H -naloxone binding and ^3H -NA uptake in various regions of rat brain

Region	^3H -naloxone binding (fmol per mg protein)		^3H -NA uptake (fmol per mg protein)	
	Controls	Lesioned	Controls	Lesioned
Cerebellum	19 ± 3	9 ± 0.6 (−46%)*	311 ± 38	97 ± 24 (−71%)*
Hypothalamus	73 ± 5	61 ± 10 (−17%)*	776 ± 102	372 ± 39 (−52%)*
Hippocampus	53 ± 5	49 ± 6 (−9%)*	474 ± 116	173 ± 17 (−60%)*
Striatum	112 ± 6	126 ± 17 (+11%)*	3,477 ± 526	4,567 ± 1,019 (+23%)*
Cortex	141 ± 5	101 ± 18 (−31%)*	450 ± 43	194 ± 17 (−57%)*

6-OHDA ($2 \mu\text{g ml}^{-1}$) dissolved in saline containing 2% ascorbic acid was unilaterally microinjected in the locus coeruleus of 180-g male Wistar rats under chloral anaesthesia. $4 \mu\text{l}$ of the solution were injected during 5 min at the following stereotaxic coordinates: P = 0.7 mm, L = 0.8 mm, H = 2.5 mm above the horizontal plane (Atlas of König and Klippel⁹). Animals were killed 15–40 d later. The ipsilateral hippocampus, striatum or hemicortex and the whole cerebellum or hypothalamus were homogenised in 10 volumes of 0.32 M sucrose using a Potter glass-Teflon homogeniser. Aliquots of the same homogenate were used for ^3H -naloxone binding and ^3H -NA uptake determinations. Binding studies were carried out with 6 nM ^3H -naloxone (9.3 Ci mmol^{-1} ; NEN) at 30 °C, in 50 mM Tris-HCl buffer, pH 7.4, according to Pollard *et al.*¹⁰ (nonspecific binding in the presence of 1 μM naloxone). Uptake studies were carried out with 17 nM ^3H -NA (15 Ci mmol^{-1} ; Radiochemical Centre) at 37 °C in bicarbonate Krebs-Ringer medium gassed with $\text{O}_2:\text{CO}_2$ (95:5) at pH 7.4 (5-min incubations) (results corrected from blanks (zero-time incubations)). Means $\pm$ s.e.m. of data from 3–8 rats.

* $P < 0.01$ as compared to respective controls (untreated rats) according to variance analysis.

† Not significant.

‡ $P < 0.05$.

cortex and cerebellum (Table 1), suggesting that opiate receptors are present on noradrenergic terminals in these regions; the absence of significant decrease in other regions does not exclude the possibility of a similar situation there but is probably due to the fact that opiate receptors on noradrenergic terminals represent only a small percentage of their total number in these areas. Similar lesion studies have led to the idea that opiate receptors are localised presynaptically on various neuronal systems in the CNS^{10,11}.

The presynaptic localisation of opiate receptors on noradrenergic neurones agrees with the conclusions reached by Garcin and Coyle¹² following perinatal administration of 6-OHDA and probably explains why opioids inhibit the depolarisation-induced release of NA from cortical and cerebellar slices^{4,5}. The discrepancy between our results and those of Kuhar *et al.*¹³ might be due to the relatively small percentage of opiate receptors on cortical noradrenergic terminals which renders their detection by lesion studies difficult.

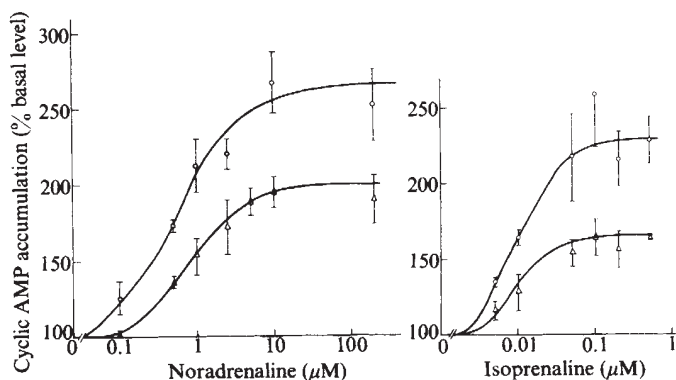
The possible development of hypersensitivity to NA in rat cortex following chronic treatment with morphine was assessed by evaluating the responsiveness of the cyclic AMP-generating system in cortical slices to a supramaximal concentration of NA¹⁴ as well as by measuring the binding of ^3H -dihydroalprenolol, a specific β -adrenergic ligand. In nine rats chronically implanted with morphine pellets for 10 d (ref. 15) the stimulation of cyclic AMP accumulation (measured in the absence of a phosphodiesterase inhibitor) was $191 \pm 19\%$, compared with $126 \pm 10\%$ in controls ($P < 0.01$), representing a 50% increase in the maximal response, without significant change in the basal level of cyclic AMP ($5.5 \pm 0.5 \text{ pmol per mg protein}$ compared with $6.4 \pm 0.3 \text{ pmol per mg protein}$ in controls). In the same animals the binding of 0.8 nM ^3H -dihydroalprenolol to cortical membranes was also significantly increased, by 19% ($34.4 \pm 1.2 \text{ fmol per mg protein}$ compared with $29.0 \pm 0.9 \text{ fmol per mg protein}$ in controls).

These effects on the cyclic AMP system were further analysed in additional groups of rats by establishing complete concentration-response curves to NA or isoprenaline in the presence of a phosphodiesterase inhibitor, isobutylmethylxanthine (Fig. 1). Clearly, in these conditions the maximal response to either NA or isoprenaline is significantly increased in morphine-treated animals (by 49% and 93%, respectively) without significant change in the EC_{50} of either agent or in the basal cyclic AMP level. These changes were paralleled by an increased number (+19%) of β -adrenergic receptors, again without change in the apparent affinity for the

^3H -ligand (Table 2). The responsiveness of the cyclic AMP system to NA remained increased two days after pellet removal and was not modified 15 h after a single 40 mg per kg injection of morphine.

Thus, rats chronically treated with morphine apparently develop a marked hypersensitivity of postsynaptic cells to adrenergic agonists, indicated by an increased responsiveness to NA and isoprenaline which can be due, at least in part, to an

Fig. 1 Effects of noradrenaline and isoprenaline on cyclic AMP accumulation in cortical slices of rats chronically treated with morphine. Male Wistar rats (160 g) were chronically treated with morphine using the procedure of Bläsing *et al.*¹⁵. Morphine pellets containing 70 mg of morphine base or placebo pellets were implanted on the following schedule: one on the 1st day, two on the 4th and three on the 7th. Rats were killed on the 11th day, 15 h after pellet removal. Cortical slices ($0.25 \text{ mm} \times 0.25 \text{ mm} \times 1 \text{ mm}$) were prepared with a McIlwain tissue chopper and preincubated for 40 min at 37 °C in a Krebs-Ringer medium pH 7.4 under a constant stream of $\text{O}_2:\text{CO}_2$ (95:5). After addition of NA (left panel) or isoprenaline (right panel) in increasing concentrations, incubations were continued for 10 min, in the presence of 0.5 mM isobutylmethylxanthine, and cyclic AMP was determined by the protein kinase binding method¹⁴. Cyclic AMP accumulation is expressed as % of basal levels ($12.8 \pm 1.1 \text{ pmol per mg protein}$ in morphine-implanted rats and $15.0 \pm 2.1 \text{ pmol per mg protein}$ in controls) and represents the means $\pm$ s.e.m. of 3–5 experiments on pooled slices from 1–3 animals (triplicate incubations). ○, Morphine treated; △, controls.



increased number of β -adrenergic receptors. Similar changes on cortical target cells occur after 6-OHDA-induced interruption of their noradrenergic inputs¹⁶, associated with an increase in the responsiveness of the cyclic AMP system to isoprenaline greater than the increase in the density of β -adrenergic receptors. In addition, an increased responsiveness of the cyclic AMP system to NA is known to develop following various pharmacological treatments resulting in an impaired noradrenergic transmission in brain^{7,8}. Therefore, in view of the decreased noradrenergic transmission elicited by morphine acting at the level of the cell bodies^{1,2} and/or by interaction with the presynaptic opiate receptors^{4,5} demonstrated by our lesion studies, it is likely that chronic administration of morphine also elicits the development of a state of 'disuse' hypersensitivity. An important consequence of this finding is that the primary effect of morphine, that is, decreased noradrenergic transmission, could be progressively compensated by the increased sensitivity of the target cells to NA. In other words, hypersensitivity to NA could well account for the development of tolerance to the actions of morphine on this system, as shown in Fig. 2.

Because (1) opiate receptors seem to be localised presynaptically on a variety of neuronal systems^{10,11}, (2) the stimulation of these receptors by opioid agents inhibits the release not only of NA^{4,5} but also of several transmitters such as dopamine¹⁷⁻¹⁹, acetylcholine¹⁹ or substance P (ref. 20) (although this action has been recently questioned in the case of dopamine⁵), (3) the development of disuse hypersensitivity seems to be a general homeostatic process in the CNS^{7,8}, the model we postulate for the long-term actions of morphine on noradrenergic systems could account for the development of tolerance to this agent in other systems. In this respect it is interesting that denervation hypersensitivity to substance P in the spinal cord has been recently described²¹ and, in view of the involvement of this system in the antinociceptive effects of opioids²⁰, this process could account for the tolerance to their analgesic actions. The fact that protein synthesis inhibitors prevent the development of tolerance to morphine³ as well as of disuse hypersensitivity²² might be attributed to the inhibition of new receptor molecule synthesis, which seems to be a common feature of the two processes.

Because the state of disuse hypersensitivity^{7,8} largely outlasts the duration of the inducing treatment^{7,8}, the same model could account for the abstinence syndrome or the symptoms of precipitated withdrawal (Fig. 2). In both cases, the abrupt interruption of a chronic treatment, restoring a normal release, produces a rebound response of the target cells bearing an excess number of postsynaptic receptors (Fig. 2).

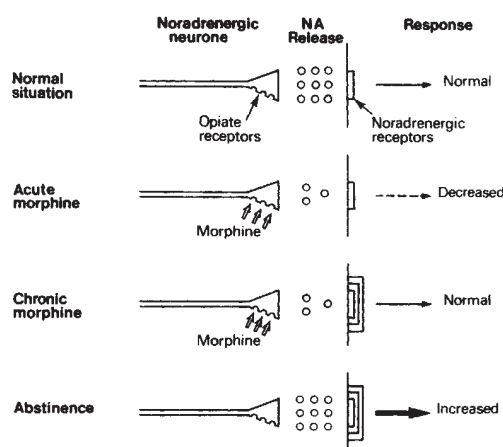


Fig. 2 Model for the effects of acute and chronic morphine treatments on noradrenergic transmission.

The model we propose for tolerance and dependence to opioids, analogous to the mechanism postulated in general terms by Collier²³ and by Jaffe and Sharpless²⁴, does not imply a change at the level of the opiate receptors themselves. This model would be consistent with the lack of change in opiate receptor binding after chronic morphine²⁵. If our model is correct, the understanding of physical dependence to opioids would require a better knowledge of the factors involved in the control of receptor synthesis and in the development of disuse hypersensitivity in the CNS.

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Table 2 ³H-dihydroalprenolol binding in cerebral cortex of rats chronically treated with morphine

	Controls	Morphine
Maximal binding (fmol per mg protein)	70.1 ± 1.7	83.3 ± 3.6* (+19%)
K _d (nM)	1.04 ± 0.05	1.07 ± 0.10†

Rat treatments were as described in the legend to Fig. 1. ³H-dihydroalprenolol binding was determined on cortical homogenates (about 20 mg tissue per ml) of individual animals. Tissues were preincubated for 15 min at 30 °C in 50 mM Tris-buffer pH 8.0. After addition of ³H-dihydroalprenolol (32 Ci mmol⁻¹, Radiochemical Centre), incubations were carried out for 20 min at 30 °C and then stopped by dilution and filtration on Whatman GF/B filters. The filters were washed twice with 15 ml of ice-cold Tris-buffer. Nonspecific binding was evaluated in the presence of 1 μM alprenolol. Saturation kinetics, determined with four concentrations of the ³H-ligand, were analysed by the plots of Dixon (apparent K_d) and Eadie (B_{max} determination). Means ± s.e.m. from five experiments.

* P < 0.01 as compared to respective controls by the Student's *t* test.

† Not significant.

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Are physiological oxygen concentrations mutagenic?

HYPERBARIC oxygen is known to be mutagenic towards *Escherichia coli*¹⁻³. But, can oxygen be mutagenic at tensions physiological to man? We have studied this question using oxygen-sensitive mutants of the histidine auxotroph strain TA100 of *Salmonella typhimurium* constructed by Ames and his coworkers as part of a test system for carcinogens⁴. We find that oxygen tensions physiological to higher animals are mutagenic towards these organisms.

S. typhimurium TA100 was cultured as described in ref. 4. Anaerobic procedures were carried out in a glove box equipped with an airlock; the main chamber was continually flushed with N₂ deoxygenated by passage over hot copper wire. Deoxygenated sterile agar medium was prepared by equilibration with filtered N₂ for 30 min at 65°C; plates were then poured in the anaerobic glove box. Anaerobic jars equipped with a hydrogen-carbon dioxide generator, Pt catalyst and oxygen indicators were used to incubate the plates. All media used to support anaerobic growth of test organisms were supplemented with 1-2% glucose. Mutations were induced by *S. typhimurium* TA100 by UV irradiation of suspensions of cells from overnight cultures. Nutrient agar (Difco) plates were inoculated at 37°C under anaerobic conditions for 4 to 7 days. These plates were replicated onto nutrient agar plates which were then incubated at 37°C, one anaerobically, the other in air. After incubation the replicas were compared. Colonies were selected from anaerobic plates which were either totally absent or grew as microcolonies on aerobically-incubated plates. The cells were then grown anaerobically in nutrient broth, frozen, and stored at -80°C. Working cultures were maintained on nutrient agar slants in an anaerobic jar at room temperature. Table 1 summarises the isolation of 25 suspected O₂-sensitive mutants; in our conditions for mutagenesis, O₂-sensitive strains were readily obtainable.

Seventeen of the suspected mutants were examined for their degree of sensitivity to oxygen. Nutrient agar plates containing 2% glucose were inoculated from an anaerobic nutrient broth culture of each mutant. Plates were incubated anaerobically or aerobically at 37°C in various oxygen concentrations, obtained by incubating the plates in an ambient atmosphere (20% O₂) or under a slow continuous stream of 1%, 5%, or 10% oxygen in N₂ with 5% CO₂. The growth of the mutants and their parent strains on the O₂-exposed plates was compared with their growth under anaerobic conditions. The degree of anaerobic growth was similar for the mutant and parent strains. Of the 17

mutants, 10 were unable to grow in an atmosphere containing 20% O₂, but did grow at lower oxygen levels (Table 2).

Five oxygen-sensitive mutants were chosen for determining the mutagenic effect of low O₂ concentrations. The mutant and parent strains were grown anaerobically in nutrient broth. The cells were concentrated by centrifugation and resuspended in Vogel-Bonner medium E. Under anaerobic conditions 0.1 ml of cell suspension (10⁷-10⁸ colony forming units, CFU) was added to 2 ml of a top agar mixture and poured over minimal medium agar plates as described by Ames *et al.*⁴; however, the top agar mixture lacked liver microsomes. One set of plates was incubated anaerobically, the other series incubated under a gentle stream of gas consisting of 5% O₂, 5% CO₂, and 90% N₂. After incubation, the revertants to histidine prototrophy were counted. Exposure to oxygen significantly increased the frequency of histidine-dependent revertants in three strains (mutants 3, 5, and 7; Table 3). Under anaerobic conditions, the frequency of mutation to histidine independence for four of the five mutants was similar to that of the parent strain. However, mutant strain 4 showed a higher reversion rate in the absence of O₂ than the parent. In mutant 8, oxygen exposure did not increase the frequency of mutation to histidine independence; instead, growth was inhibited as indicated by the presence of numerous microcolonies in the background. Reversion to histidine prototrophy was confirmed by transferring selected colonies to minimal medium lacking histidine.

Table 2 Effect of O₂ on the growth of suspected O₂-sensitive mutants of *S. typhimurium* TA100

Organism	Atmospheric O ₂ concentration				
	21%	10%	5%	1%	0%
<i>S. typhimurium</i>					
TA100 (parent)	N	N	N	N	N
Mutant 1	-	N	N	N	N
Mutant 2	-	+	N	N	N
Mutant 3	-	+	N	N	N
Mutant 4	-	+	+	N	N
Mutant 5	-	+	+	+	N
Mutant 6	-	+	+	+	N
Mutant 7	-	+	+	+	N
Mutant 8	-	-	N	N	N
Mutant 9	-	-	+	+	N
Mutant 10	-	-	-	-	N

Plates were either incubated anaerobically at 37°C for 3 to 4 days or aerobically with varying O₂ concentrations at 37°C for 2 days. The results represent at least two experiments performed in duplicate. N, normal growth, similar to growth of parent; +, growth as microcolonies; -, no visible growth.

Table 1 Selection of oxygen-sensitive mutants from *S. typhimurium* TA100 after exposure to UV irradiation

Expt	CFU ml ⁻¹	Survival after UV irradiation (%)	No. of colonies examined	No. of suspected O ₂ -sensitive mutants
A	1.4 × 10 ⁷	47	2134	3
B	1.23 × 10 ⁵	15	NC*	4
C	1.52 × 10 ⁵	6	1106	2
			996	3
			1087	1
D	9.5 × 10 ⁵	5	NC	2
			1073	4
			1245	3
			NC	3

* NC, not counted.

The 10 mutants isolated from *S. typhimurium* TA100 were oxygen sensitive because surface growth was absent after incubation in 20% O₂. But these mutants were only moderately anaerobic. Surface growth on nutrient agar could take place at 0.5%-10% O₂, depending on the mutant. Similar variations in oxygen sensitivity occur among naturally occurring anaerobic bacteria and suggest that more than one mechanism may be responsible for the O₂ toxicity⁵.

A steady state may exist in respiring cells between the rate of production of reactive forms of oxygen generated by autoxidation or leaked from normal oxygen metabolising pathways, and their rate of degradation⁶. Alterations in this steady state could lead to oxygen sensitivity; and oxygen-sensitive mutants have been isolated which are deficient in superoxide dismutase^{6,7}. The mechanism of O₂-dependent back mutation to histidine prototrophy in our oxygen-sensitive strains of *S. typhimurium* TA100 is unknown; but a base-pair substitution is probably involved⁴.

Table 3 Mutagenic effect of O₂ with respect to reversion to histidine prototrophy by O₂-sensitive mutants of *S. typhimurium* TA100

Organism	Mutation frequency ($\times 10^{-6}$)	
	-O ₂	+5% O ₂
<i>S. typhimurium</i> TA100 (parent)	0.25 $\pm$ 0.04	0.64 $\pm$ 0.18
	0.40 $\pm$ 0.10	0.71 $\pm$ 0.12
	1.10 $\pm$ 0.60	2.23 $\pm$ 0.06
Mutant 5	0.66 $\pm$ 0.11	>8.8
	0.35 $\pm$ 0.10	>14.3
	0.31 $\pm$ 0.02	15.30 $\pm$ 0.90
Mutant 3	0.36 $\pm$ 0.10	>2.3
	0.60 $\pm$ 0.11	>5.2
	0.11 $\pm$ 0.03	2.70 $\pm$ 0.30
Mutant 7	0.28 $\pm$ 0.08	>2.4
	0.42 $\pm$ 0.07	>5.7
	0.14 $\pm$ 0.02	2.30 $\pm$ 0.30
Mutant 4	10.10 $\pm$ 0.60	13.40 $\pm$ 1.60
	16.00 $\pm$ 4.00	30.00 $\pm$ 2.00
	25.00 $\pm$ 3.00	34.00 $\pm$ 1.00
Mutant 8	0.59 $\pm$ 0.05	0.16 $\pm$ 0.01*
	0.98 $\pm$ 0.07	0.77 $\pm$ 0.17*
	0.87 $\pm$ 0.09	0.65 $\pm$ 0.11*

Agar plates were incubated anaerobically at 37 °C for 3 to 5 days or under 5% O₂ at 37 °C for 2 days. The results of three experiments are presented, each result being the average of three or four agar plates.

* Numerous microcolonies present in the background.

Our results show that exposure of oxygen-sensitive cells to concentrations of oxygen physiological for mammals can lead to mutation. According to Fridovich⁶ oxygen toxicity is normally held in check by a balance among the rates of formation and destruction of reactive forms of oxygen. This may mean that oxygen mutagenicity is improbable, but not impossible, in normal aerobic mammalian cells; but higher rates of formation of reactive forms of oxygen or lower rates of their destruction, analogous to processing occurring in oxygen-sensitive forms of *S. typhimurium* TA100, could lead to significant rates of mutagenesis along with the molecular pathologies arising from mutation.

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Mutagenicity of thymidine to cultured Chinese hamster cells

THYMIDINE starvation is known to be both toxic and mutagenic to various prokaryotes, including *Escherichia coli*^{1,2}, *Bacillus subtilis*³ and bacteriophage T₄ (refs 4-6). Base excess, as well as base starvation, can also be mutagenic. For instance, Bernstein *et al.*⁵ showed that a superabundance of exogenous thymidine (4 mM) produced an increased reversion rate for some T₄ amber mutants. In mammalian cells a superabundance of exogenous thymidine is toxic, perhaps by inhibiting DNA synthesis⁷⁻⁹. The mechanism for these effects may be related to the observations that dTTP inhibits the enzymatic reduction of CDP to dCDP by ribonucleoside diphosphate reductase¹⁰ and also that thymidine reduces the dCTP pools of Chinese hamster cells to 90% of normal¹¹. We report here that a superabundance of exogenous thymidine is both toxic and highly mutagenic to V-79 Chinese hamster cells but that hydroxyurea, an inhibitor of ribonucleoside diphosphate reductase, is toxic but not mutagenic.

The toxicities of thymidine, deoxycytidine and hydroxyurea for V-79 Chinese hamster lung (CHL) cells are shown in Fig. 1. Hydroxyurea is much more toxic than thymidine on an equi-

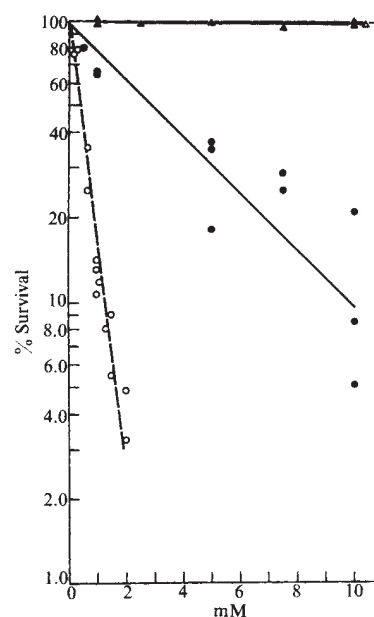


Fig. 1 Toxicity of thymidine, deoxycytidine and hydroxyurea for V-79 CHL cells. 300 exponentially growing V-79 cells were inoculated into 10 ml of complete medium (CM) (α -minimal essential medium¹²), minus purines and pyrimidines, containing 5% fetal calf serum (Flow) and 25 mM HEPES in 100-mm Falcon plastic Petri dishes. The cells were allowed to attach to the dishes at 37 °C in a 7.5% CO₂/air incubator. Four hours after inoculation the medium was replaced with either fresh CM for controls or fresh CM with the appropriate concentration of drug. Experimental and control media were replaced with fresh CM 24 h later. After one week of incubation the colonies were rinsed with buffered saline solution, fixed in methanol for 10 min, stained with Giemsa and counted. A colony was defined as more than 64 cells in close proximity. The plating efficiency of the control cells in CM was between 83 and 94%. The number of colonies surviving after treatment is normalised to the plating efficiency of the controls. Each point represents the mean of three independent plates obtained in one experiment. Symbols: ○, hydroxyurea; ●, thymidine; ▲, deoxycytidine; △, 10 mM thymidine plus 3 mM deoxycytidine.

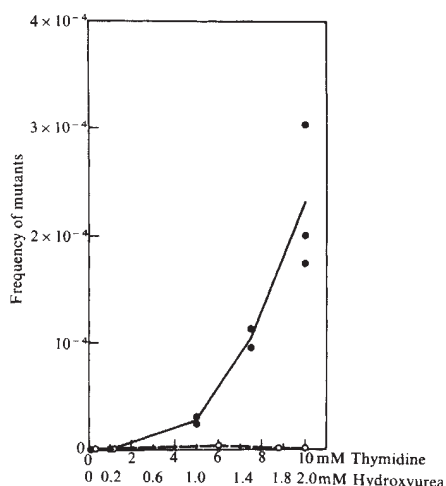


Fig. 2 Linear plot of mutant frequencies induced in CHL cells by thymidine and hydroxyurea as a function of concentration. The frequency of mutants in V-79 cells after drug treatment was measured as described in Bradley and Sharkey¹². Briefly, stock cells were treated with hypoxanthine, aminopterin and thymidine (HAT) medium for one week to remove pre-existing mutants. After recovery from HAT these cells were trypsinised and seeded into 150-cm² flasks in 50 ml of CM. Two days later, when the cells ($1-3 \times 10^7$) were in log phase, they were treated with thymidine or hydroxyurea for 24 h. After treatment the medium was removed and replaced with fresh CM. Two days later cells from the treated and control flasks were subcultured into 150-cm² flasks at 2×10^6 cells per flask to allow further time for mutations to be expressed. The flasks were incubated for at least another 3 d until they were about to become confluent. This 5-7 d expression time gave maximal and stable induced mutant frequencies. The fraction of mutant cells in the population at this time was assayed by seeding a total of 2.7×10^6 treated and recovered cells among nine 100-mm Petri dishes (3×10^5 cells per dish) in 15 ml of CM containing 6-thioguanine ($11 \mu\text{g ml}^{-1}$). At the same time, the plating efficiency of these cells was determined by seeding 300 cells each in six 100-mm Petri dishes in CM. All plates were incubated one week before fixing and staining. The mutant frequency was calculated by dividing the number of mutant colonies by the plating efficiency. The background mutant frequencies ($1-4 \times 10^{-6}$) were subtracted from the values plotted here. Note the change in scale for the concentrations of hydroxyurea and thymidine. Symbols: ●, thymidine; ○, hydroxyurea.

molar basis. Deoxycytidine, by itself, is not toxic at concentrations up to 10 mM. However, 3 mM deoxycytidine completely reversed the toxicity of 10 mM thymidine.

The effect of thymidine and hydroxyurea on the frequency of mutants induced in CHL cells is shown in Fig. 2. Resistance to 6-thioguanine was used as a marker for inactive hypoxanthine-phosphoribosyl transferase and as an assay for mutant colonies. The method is briefly described in the legend to Fig. 2. The number of 6-thioguanine resistant, presumably mutant, colonies begins to increase at concentrations greater than 10^{-3} M thymidine. At higher concentrations the number of mutants induced rises rapidly. At survival values $>65\%$ (10^{-5} – 10^{-3} M thymidine) no dose-dependent increase occurs in the mutant frequency nor are the values significantly different from the control values. These data suggest that toxicity may be

Table 2 Effects of deoxycytidine with and without thymidine on the frequency of mutants in CHL cells

Deoxynucleotide	% Survival	Experimental mutant frequency	Control mutant frequency
10^{-3} M dC	100	3.34×10^{-6}	4.33×10^{-6}
3×10^{-3} M dC	103	2.55×10^{-6}	
10^{-2} M dC	100	3.24×10^{-6}	
10^{-2} M dT	18	1.01×10^{-4}	
3×10^{-3} M dC + 10^{-2} M dT	103	1.03×10^{-5}	

induced at doses of thymidine that do not produce mutations. Hydroxyurea, on the other hand, was not mutagenic at any concentration tested (Fig. 2, Table 1) spanning a wide range of toxicities.

The finding that deoxycytidine itself is not mutagenic at concentrations of up to 10 mM (Table 2) and at 3 mM will greatly suppress the induction of mutants by 10 mM thymidine suggests that thymidine is mutagenic because it reduces the deoxycytidine pool and that resupplying this pool with exogenous deoxycytidine reverses the effect. Low concentrations of deoxycytidine are known to increase dCTP pools in Chinese hamster cells¹¹, although other unknown mechanisms of action might also be important.

As hydroxyurea inhibits DNA synthesis but is not mutagenic, it is clear from these data that inhibition of DNA synthesis by itself is not necessarily mutagenic. Thymidine also inhibits DNA synthesis⁷⁻⁹, is toxic, and, as shown here, is mutagenic. However, 2.5 mM thymidine creates a $>1,000$ -fold increase in the ratio of the dTTP pool to the dCTP pool^{11,13}, while maintaining nearly constant ratios in the dATP and dGTP pools^{11,13}. As DNA synthesis continues slowly in 10 mM thymidine¹¹, it seems likely that thymidine would be misincorporated for deoxycytidine in conditions of such large dTTP/dCTP pool imbalances. Such a substitution would be expected to produce a G:C $\rightarrow$ A:T transition and thus a mutation. In bacteriophage T₄ thymineless mutagenesis has been shown to produce the opposite, A:T $\rightarrow$ G:C, transition⁶. The finding that deoxycytidine reverses the toxicity and mutagenicity of thymidine argues in favour of such a base imbalance hypothesis. On the other hand, hydroxyurea reduces the pool sizes of dATP and dGTP without creating such large changes in the ratios of one deoxynucleotide to the others^{13,14}. These simultaneous reductions may be toxic but not mutagenic, although other mechanisms of action may contribute to hydroxyurea activity. Our hypothesis based on these data is that for mammalian cells large pool imbalances can cause mutation because of base substitution, that decreased pool sizes during DNA synthesis can be toxic, and that decreasing DNA synthesis is not by itself necessarily either toxic or mutagenic.

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Table 1 Lack of effect of hydroxyurea on mutagenesis in CHL cells

	Hydroxyurea concentration (mM)	% Survival	Experimental mutant frequency	Control mutant frequency
Expt 1	0.1	104	2.40×10^{-6}	3.71×10^{-6}
	0.3	80	3.29×10^{-6}	
	1.0	13	3.59×10^{-6}	
Expt 2	1.0	10	5.27×10^{-7}	1.09×10^{-6}
	1.5	5.4	5.76×10^{-6}	3.27×10^{-6}
	2.0	3.3	5.81×10^{-7}	5.51×10^{-7}

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Mutagen-oligonucleotide complexes with a bulged base as models for frameshift mutation

ACRIDINES and ethidium are frameshift mutagens¹ which intercalate into DNA and RNA², and into minihelices^{3,4} made of complementary dinucleotides. However, this intercalation does not provide an explanation of their mutagenic action. Frameshift mutagenesis requires a bulge of one or two bases in either the parent strand (a deletion mutation), or in the newly synthesised strand (an addition mutation). That is, for frameshift mutation to occur, some bases must remain unpaired and outside the normal double helix during replication. We have prepared minihelices containing such bulged bases stabilised by ethidium. Nuclear magnetic resonance (NMR) studies reported here provide strong evidence that a mixture of the oligonucleotides GpUpG plus CpC in the presence of ethidium produces a minihelix containing two G·C base pairs with the uracil base bulged outside the helix and the ethidium intercalated. Similarly, CpUpG forms minihelices in the presence of ethidium containing bulged bases on both strands. These complexes provide models for the mutagenic action of ethidium.

Krugh *et al.* have extensively studied the spectroscopic properties of dinucleoside phosphates and ethidium^{3,4}. NMR, ultraviolet (UV) absorption, circular dichroism (CD) and fluorescence studies show that complementary dinucleoside phosphates form minihelices with intercalated ethidium. X-ray studies of crystals of these complexes containing iodo-CpG and iodo-UpA provide their detailed geometry in the solid phase⁵. We have measured the proton magnetic resonance of dinucleoside phosphates, trinucleoside diphosphates and their mixtures in the presence of ethidium. Not only have we studied the aromatic protons of the ethidium and the bases, but the sugar protons have also been analysed. This provides new information about the conformations of the sugars in the complex in solution.

When CpG and ethidium are mixed, large upfield shifts of all the protons of the phenanthridinium ring of ethidium occur^{3,4} (Table 1). These shifts are too large to be accounted for by stacking ethidium on one base or base pair⁶; at least two base

Table 1 Upfield chemical shifts of ethidium in the presence of oligonucleotides

Proton	δ (free ethidium) - δ (ethidium in complex) in p.p.m.			
	CpG*	CpUpG†	GpUpG‡	GpUpG + CpC§
H1	0.58	0.52	0.24	0.45
H2	0.92	0.66	0.24	0.68
H4	1.22	0.75	0.21	0.61
H7	1.05	0.63	0.28	0.62
H9	0.85	0.66	0.22	0.56
H10	0.58	0.49	0.25	0.38

Shifts are reported at 10 °C in D₂O solution containing EDTA. Spectra were recorded on a Bruker HXS 360-MHz spectrometer (Stanford Magnetic Resonance Laboratory).

*5 mM CpG + 2.5 mM ethidium.

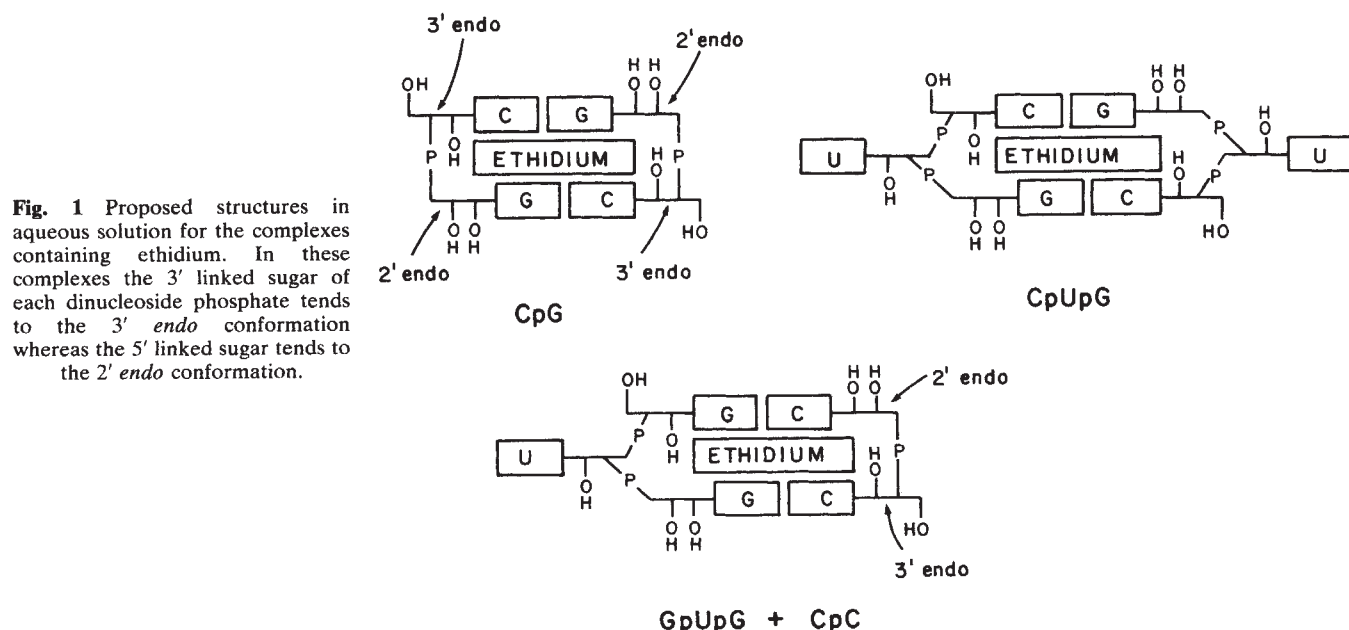
†3.2 mM CpUpG + 1.6 mM ethidium.

‡2 mM GpUpG + 2 mM ethidium.

§2 mM GpUpG + 2 mM CpC + 2 mM ethidium. At these concentrations the complex is not fully formed.

pairs (one on each side of the ethidium) are required. The conclusion that ethidium is intercalated between the two G·C base pairs of a minihelix has been amply confirmed by other spectroscopic methods^{3,4}. Using the same criteria (Table 1) we conclude that the trinucleoside diphosphate, CpUpG, forms a minihelix of two G·C base pairs with ethidium intercalated. The maximum ring-current shift of a stacked uracil is only 0.1 p.p.m.⁶, therefore, in the complex the uracil bases must be moved away from the ethidium, thus allowing direct stacking with each of the G·C base pairs. Similar arguments apply to the complex of GpUpG, CpC and ethidium. For this system NMR measurements of GpUpG plus ethidium produce upfield shifts for the ethidium of less than 0.28 p.p.m. (Table 1); CpC plus ethidium gives shifts of less than 0.1 p.p.m. However, at the same concentrations GpUpG plus CpC plus ethidium causes upfield shifts for the ethidium of from 0.38 to 0.62 p.p.m. This further supports the special nature of the interaction of the base pairs with ethidium. NMR studies of trinucleoside diphosphates with the sequence purine-pyrimidine-purine C (H.L. and I.T. unpublished) show that in these molecules the pyrimidine tends to bulge out, allowing the purines to stack. This favours a similar conformation in the minihelices stabilised by ethidium.

Temperature dependence of the ethidium chemical shifts yields sigmoid curves for the three systems studied. For the



concentrations given in Table 1, CpG plus ethidium showed a 'melting temperature,' T_m , of about 50 °C, CpUpG plus ethidium had a T_m near 35 °C, and GpUpG, CpC plus ethidium a T_m below 20 °C.

The conformations of the ribose parts of the oligonucleotides can be determined from their coupling constants⁷. In particular, $J_{1'2'}$ is directly related to the % 3' *endo* conformation: % 3' *endo* = 10 (10 - $J_{1'2'}$). Although there is a broadening of all the NMR lines on complex formation, the coupling constants indicate a characteristic change in conformation of the dinucleoside phosphates in the complex. The free oligonucleotides show a mixed puckering of each ribose with roughly 60% 3' *endo*, but in the complex the amount of 3' *endo* increases in the 3' linked nucleotide whereas the 5' linked nucleotide becomes mainly 2' *endo*. At 40 °C free CpG showed 62% 3' *endo* for Cp and 52% 3' *endo* for pG; in the presence of ethidium such that about half the dinucleoside phosphate was complexed, the $J_{1'2'}$ values indicate a 3' *endo* conformation for Cp and 2' *endo* for pG. The CpC in the GpUpG:CpC:ethidium complex also tends to 3' *endo* for the Cp and 2' *endo* for pC. The $J_{2'3'}$ and $J_{3'4'}$ coupling constants also support this conclusion. This 3' *endo*-2' *endo* conformation has also been found for intercalated ethidium complexes in the solid state⁸. We did not see evidence of changes in sugar puckering of CpUpG or GpUpG in the ethidium complexes.

The coupling constants $J_{4'5'}$, $J_{4'5''}$, $J_{5'P}$, $J_{5''P}$, and $J_{3'P}$ in the proton NMR spectra monitor the conformations of the exocyclic linkages (C4'-C5', C5'-O5' and C3'-O3' bonds). None of these coupling constants showed large changes relative to the free oligonucleotides for either the dinucleoside phosphates or trinucleoside diphosphates. Changes in conformation caused by rotation about the 3'O-P and 5'O-P bonds would not be seen, as these changes are not monitored by any proton coupling constants.

Complexes of ethidium and dinucleoside phosphates containing deoxyribose sugars in both strands, or in hybrid (one strand ribose-one deoxyribose) helices, show similar behaviour for the sugar-phosphate backbone. That is, a 3' *endo*-2' *endo* conformation is approached in the complex.

When ethidium is mixed with complementary dinucleoside phosphates a red shift in its absorption occurs, the fluorescence quantum yield increases and circular dichroism is induced in the non-chiral ethidium^{3,4}. These same phenomena occur with ethidium plus CpUpG, and ethidium plus GpUpG, CpC. In both systems a strong positive circular dichroism band is induced in the 300-365 nm region and a weak negative CD occurs from 365 to 560 nm. A mixture of GpUpG plus ethidium shows only a weak induced band in the 300-365 nm region. The absorption and induced CD have been used to study the stoichiometry of the complexes with the bulged bases (A. Pardi, unpublished). In excess oligonucleotide the stoichiometry is 2CpUpG:1 ethidium and 1GpUpG:1CpC:1 ethidium. At higher ratios of ethidium to nucleotide the stoichiometry indicates that more ethidium is incorporated into the complex, presumably stacked on the outside of the minihelices and on the bulged uracil bases.

The NMR, CD and stoichiometry results are all consistent with the structures for the complexes proposed in Fig. 1. Work is continuing to determine more detailed structures and also to study deoxyribo-oligonucleotides. If ethidium does cause frameshift mutations by intercalation into transient bulges formed during replication, recombination or repair, it is important to know if ethidium preferentially binds at a bulge rather than a perfect helix. Thermodynamic studies of the complexes can answer this question.

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Globin RNA sequences in human leukaemic peripheral blood

EVIDENCE for the involvement of more than one cell line and even interconversion of different forms of human leukaemia suggests that the neoplastic transformation occurs in a common stem cell¹. Although erythroid leukaemias are very rare in humans, disturbances in haemopoiesis or haemoglobin synthesis² are common in leukaemia, suggesting the possible involvement of erythroid precursors. We show here, by the use of sensitive cDNA (complementary DNA)-RNA hybridisation, that in a substantial number of human leukaemias globin RNA sequences can be demonstrated in nuclear RNA prepared from highly purified leukaemic cell populations.

Leukaemic cells were prepared from peripheral blood by a two-step procedure including spontaneous differential sedimentation of red cells followed by centrifugation on Ficoll-Paque density gradients. By this method we observed that contamination of leukaemic cells by red cells or morphologically identifiable red cell precursors was always <1 in 10,000. Nuclear RNA was extracted from purified nuclei³ and hybridised to human globin complementary DNA (cDNA_{αβ}).

Figure 1 shows the hybridisation of cDNA_{αβ} with total nuclear RNA extracted from purified leukaemic cells of a patient with chronic myelogenous leukaemia (CML), blastic phase (case 4). The RNA protects almost all the sequences of the cDNA from digestion by the single strand specific S₁ nuclease; from the slope of the hybridisation it can be calculated¹⁰ that globin sequences represent at least 1 part out of 250,000 by weight of the total nuclear RNA extracted from leukaemic cells.

Several control experiments were carried out. The possibility that globin RNA sequences were due to contaminating reticulocytes was ruled out by the following experiment: after sedimenting erythrocytes free of leukaemic cells, red cells were mixed back with their own plasma and with an equal mass (relative to the leukaemic cells) of rat ascites tumour cells which were then purified as above. Figure 1a shows that globin sequences are almost absent from the nuclear RNA extracted from these cells (<1 part in 3 million).

The level of hybridisation observed cannot be explained by contaminating erythroblasts which are known to be present in the peripheral blood of some leukaemic patients; indeed, the erythroblasts contaminating the final leukaemic cell preparation were always less than 1 out of 10,000 as judged by morphological examination. This extremely low level reflects the fact that erythroblasts, when present in the peripheral blood of the leukaemic patients, are mostly mature forms which are lost during the purification procedure (see legend to Fig. 1). Furthermore, mature erythroblasts contain very low amounts of nuclear globin sequences (as judged by hybridisation experiments carried out on fractionated bone marrow cells or thalassaemic peripheral erythroblasts, unpublished results) so that it can be calculated that up to 1-2% contamination with orthochromatic erythroblasts or 0.4-0.6% with immature

forms should be present in our final preparation to give the observed results. However, to obtain more direct evidence, we carried out reconstruction experiments by mixing increasing amounts of group-compatible, erythroblast-rich blood from a β -thalassaemic splenectomised patient with leukaemic blood. The relative proportion of erythroblasts to leukaemic cells was 1, 4 and 8% in three different mixtures. Following purification of the leukaemic cells (one case of AML and one of CLL) nuclear RNA was extracted and hybridised to cDNA $_{\alpha}$ and cDNA $_{\beta}$. No hybridisation could be observed at an RNA/cDNA ratio of $3-4 \times 10^5$ to 1, which allows easy detection of globin sequences in the positive cases (not shown). These results indicate that even a very large proportion of erythroblasts cannot account for the observed results.

A final possibility was that morphologically unidentifiable erythroid precursors (BFU 11,12) in the amount normally present in the peripheral blood, contain in their nuclei enough globin RNA sequences to account for the observed hybridisation; this possibility was ruled out by measuring the level of globin sequences in nucleated cells from blood of nonleukaemic individuals. No hybridisation was observed in the nuclear RNA prepared from leukocytes of a cancer patient with a leukaemoid reaction, nor with the RNA extracted from the nucleated cells present in a volume of blood 10 times higher than that required to saturate the cDNA probe in positive cases (Fig. 1c).

Figure 1b shows that purified cDNA $_{\alpha}$ and cDNA $_{\beta}$ hybridise equally well to the leukaemic RNA, indicating that both

normal globin RNAs are represented. In addition, melting point determination (inset) confirms the specificity of the hybridisation.

Table 1 summarises the results obtained in 22 different cases of both chronic and acute leukaemia. In five cases (all myelogenous leukaemias) we observed high hybridisation levels (1.8–7 p.p.m. corresponding to about 10–40 globin RNA molecules per cell taking an average RNA content per nucleus of 2 pg) which compare well with the amount detected in the erythroleukaemia case studied (12 p.p.m., about 70 molecules per nucleus). In the remaining cases, globin RNA was either absent or present at low levels, so that the percentage of globin sequences in RNA samples had to be extrapolated. Although unambiguous positivity seems to occur only in myelogenous leukaemias, the number of cases examined so far is too low to draw definitive conclusions.

Thus, although the detection of globin RNA sequences at a low level in the nuclei of peripheral leukaemic cell populations is unexpected, the above control experiments exclude artefacts, and other possible causes of hybridisation. Moreover, the RNA sequences hybridising to globin cDNA completely protect the probe and form a well matched hybrid thus confirming their identity with the true globin RNA sequences.

Humphries *et al.*⁹ found that in mice globin RNA, sequences are found at a low level in non-erythroid cells and speculate that all genes may be transcribed in every tissue. We find this explanation unlikely in our case for in several experiments (Fig. 1c) no globin sequences could be found at RNA/cDNA ratios

Fig. 1 Nuclear RNA was prepared from nucleated cells in the peripheral blood by a three-step procedure. Heparinated blood was allowed to sediment at room temperature to separate red cells from plasma; the sedimentation step removes >98% of red cells and mature erythroblasts (if present), leaving >80% of white cells in the upper phase. The cells and plasma were washed by centrifugation with 0.9% NaCl, the pellet resuspended with 5 volumes of Ficoll-Paque (Pharmacia) and the resulting suspension centrifuged at room temperature, 2,000g for 10 min. Usually, a single centrifugation results in the complete removal of residual erythrocytes and mature erythroblasts; if necessary, this step was repeated until red cell contamination was less than 1 out of 10,000 cells. Nuclei were purified by the citric acid procedure³ and nuclear RNA prepared as described by Lanyon *et al.*⁴ or by a modified Hirt⁵ procedure (A.M.G. and R.A. Weinberg, unpublished); nuclei were dissolved in 0.25 M NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 1% SDS, kept in ice for 3–4 h and spun at 20,000 r.p.m., 4°C, 30 min in a JA 20 Spinco rotor, leaving in the supernatant the RNA, which was then extracted with phenol-chloroform and precipitated with ethanol. Both methods give comparable results. Each RNA sample was titrated with human globin cDNA⁶ of specific activity 12,000 c.p.m. ng⁻¹ at a counting efficiency of 30%. cDNA $_{\alpha}$ purified essentially as described by Williamson⁷, was 85–90% pure; cDNA $_{\beta}$ was about 80% pure. In the hybridisation conditions used plateau levels are reached at 75–80% with cDNA $_{\alpha\beta}$ and cDNA $_{\beta}$, and 90% with cDNA $_{\alpha}$. A constant amount of cDNA (15–30 pg) was hybridised with increasing amounts of RNA in a volume of 1 μ l of 50% formamide, 0.5 M NaCl, 25mM HEPES buffer pH 6.8, 1 mM EDTA, containing *Escherichia coli* RNA (2.5 mg ml⁻¹). Samples were sealed in silicon-coated glass capillaries and incubated at 43°C for 12–14 d. The percentage of cDNA hybridised was determined by assay with single strand specific S₁ nuclease, followed by perchloric acid precipitation of hybridised material^{4,6}. a, cDNA $_{\alpha\beta}$ was hybridised to RNA extracted from leukaemic cells (CML, blastic phase, case 4, ●) or from rat Yoshida ascites (○) added back to the same blood reconstituted from erythrocytes and white cells free of plasma. The recovery of RNA from the leukaemic and ascites cells was identical, allowing direct comparison of hybridisation results. b, The same leukaemic RNA as in a was hybridised with purified cDNA $_{\alpha}$ (▲) and cDNA $_{\beta}$ (△). The inset shows the melting profile⁹ of normal globin 9S RNA and leukaemic RNA hybridised to cDNA. A total of 300 pg of cDNA was hybridised in 14 individual capillaries (1 μ l per sample) with a total of 1 ng of 9S RNA (●) or 230 μ g of leukaemic RNA (○) for 12 d at 45°C. At the end of the incubation the capillaries were heated at the indicated temperature, quickly cooled in ice, diluted and assayed for double stranded material as above. c, cDNA $_{\alpha\beta}$ was hybridised to nuclear RNA extracted from cases 12 (x) and 5 (▲), from a leukaemoid reaction (■) and from nonleukaemic control patients with high erythrocyte sedimentation rate and normal white cell count. Cells from the two normal cases (●, ○) were isolated after addition of ascites cells as a carrier, to a final cell concentration identical to that found in cases 5 and 10.

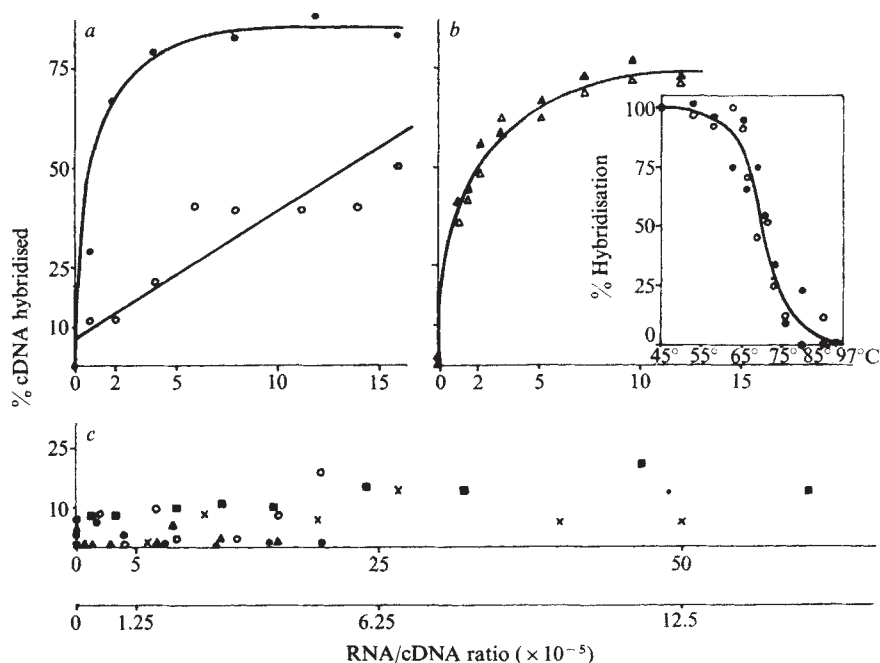


Table 1 Globin RNA content in nuclear RNA from different patients

Patient no.	Diagnosis	Presence of globin RNA	Nuclear RNA*
1	CML, blastic phase	±	1.3†
2	CML	—	0.25†
3	CML	+	7
4	CML, blastic phase	+	4
5	CML	—	0
6	CML	+	3.4
7	AML	+	1.8
8	AML	±	1.25
9	AML	+	4
10	AML	—	0.25†
11	AML	—	0
12	CLL	—	0
13	CLL	±	1†
14	CLL	—	0
15	CLL	—	0
16	CLL	—	0
17	CLL	±	1†
18	ALL	—	0
19	ALL	—	0.1†
20	ALL	—	0.1†
21	ALL	—	0
22	EL	+	12

CML, chronic myelogenous leukaemia; AML, acute myelogenous leukaemia; CLL, chronic lymphocytic leukaemia; ALL, acute lymphoblastic leukaemia; EL, erythroleukaemia. Diagnosis was by standard criteria. Blood was collected only from untreated patients with a leukocyte count $> 30,000 \mu\text{L}^{-1}$, high erythrocyte sedimentation rate and haemoglobin levels $> 10 \text{ g dl}^{-1}$.

* Percentages of globin sequences in RNA samples (expressed as p.p.m. by weight) were determined from the slope of the hybridisation according to Young *et al.*¹⁰.

† Extrapolated values due to incomplete cDNA hybridisation.

which would allow detection of less than one globin RNA molecule per nucleus. Thus, the presence of globin sequences is not a regular feature of leukaemia. A second observation relevant to our results is the ectopic production of tissue-specific proteins by tumours arising from different cell lines; most interesting is the recent report that chicken fibroblasts start transcribing globin genes following transformation by Rous sarcoma virus.¹³

An additional explanation is that leukaemia might involve a pluripotent precursor capable of differentiation into both the white cell and erythroid compartment, and expressing at some stage of its development globin RNA sequences. In fact, cytogenetic and clinical evidence suggests that, at least in myelogenous forms, the defect lies in a stem cell common to all bone marrow cell lines:¹ our biochemical findings are in keeping with this hypothesis.

At present, we have no evidence about whether globin gene expression occurs in the whole cell population or in a few cells only. Recently, erythropoietin-responsive red-cell precursors^{11,12} have been described in the peripheral blood of normal human subjects: if these cells transcribe globin genes and their number were increased in leukaemia as compared to the amount normally present in the normal individuals, our results might find an additional explanation.

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DNA phosphotriesters as indicators of cumulative carcinogen-induced damage

RECENT studies on phosphotriesters induced in DNA *in vivo* by several potent chemical carcinogens have suggested that these lesions are chemically stable and that they are not eliminated by cellular DNA-repair systems^{2–3}. It seemed possible therefore that in long-term carcinogenesis studies the accumulation of phosphotriesters might be used as a measure of the extent of damage to DNA. In a preliminary study rats have been fed continuously with either dimethylnitrosamine or diethylnitrosamine in the drinking water (0.1–5 p.p.m., w/v) and significant concentrations of methyl or ethyl phosphotriesters have been detected in tissue DNA even at the lowest dose.

In the rat, short-term treatment with high doses of dimethylnitrosamine has generally produced tumours of the kidney⁴, whereas long-term treatment with low doses (for example, 2–50 p.p.m. in the solid food⁵) usually led to the appearance of tumours of the liver. In long-term feeding experiments, diethylnitrosamine at low doses (for example, 0.5–100 p.p.m. in the drinking water⁶) induced liver tumours, whereas high doses (100–280 mg per kg body weight) delivered in one or a few doses produced tumours of the oesophagus and kidney as well as of the liver⁷.

In the experiments with dimethylnitrosamine the distribution of phosphotriesters in DNA of lung, liver and kidney found in the drinking experiments has been compared with the distribution observed after injection of single, comparatively large doses.

The concentrations of methyl phosphotriesters observed in DNA after the injection of 2–20 mg per kg dimethylnitrosamine are recorded in Table 1. It may be noted that previous experiments had confirmed that the major organs involved in the metabolism of this compound were the liver, kidney and lung, and that metabolism did not occur, for example, in spleen, thymus or brain³. The data of Table 1 suggest that the greater part of the metabolism of dimethylnitrosamine occurs in the liver, but that, at the doses used, an appreciable fraction of the unchanged compound circulates through the body and is metabolised in kidney and lung.

Results obtained from the experiment in which the compound was fed in the drinking water are shown in Table 2. At all the doses used, significant amounts of methyl phosphotriester were eventually induced in the DNA of the liver. At the highest dose (5 p.p.m.) significant and increasing amounts of phosphotriester were observed in DNA of lung. At the lower doses the data on lung DNA were inconclusive, negative results appearing with significant positive results in no obvious sequence. In the DNA from kidney, however, in only

Table 1 Distribution of methyl phosphotriesters in DNA of different tissues of rat after a single intraperitoneal injection of dimethylnitrosamine

Injected dose		Methyl phosphotriesters observed per 10 ⁶ nucleotides		
(mg per kg)	(mg per rat)	Liver	Kidney	Lung
2	0.25	9.9(1.0)	9.0(1.4)	4.2(1.2)
5	0.63	44.3(2.3)	12.7(1.0)	12.6(4.9)
10	1.25	63.1(2.8)	11.6(1.8)	8.3(1.6)
20	2.5	129.5(9.6)	26.3(1.8)	23.4(1.4)

Female, Chester Beatty, hooded rats were injected i.p. with the nitrosamine dissolved in 0.2–0.5 ml physiological saline. The animals were killed 24 h later and DNA extracted from liver, kidney and lung using the phenol method. The concentration of methyl phosphotriesters in the DNA was determined by the differential alkaline hydrolysis method as described previously^{11,12}. Each line of data refers to experiments with DNA from the tissues of one rat. The data give the number of breaks produced in a DNA chain of 10⁶ nucleotides by hydrolysis of the phosphotriester present. What exact proportion of phosphotriesters hydrolyse to give chain breaks as opposed to simple loss of the alkyl group is not yet known but breaks are probably formed about two-thirds of the time¹³. For comparative purposes this uncertainty is not important. The figures in brackets give the s.d. of each estimate calculated from the errors involved in the estimates of the two sedimentation coefficients¹².

three out of eighteen experiments were significant positive results ($P < 0.05$) observed.

Using these criteria the general conclusion seems to be that, under this method of administration of the carcinogen, practically all the compound is metabolised in the liver. However, particularly at the highest dose, some unchanged compound may reach the lungs. The lack of any apparent reaction with DNA in kidney accords with the observations that long-term feeding of dimethylnitrosamine does not tend to induce kidney tumours.

Table 2 Concentration of methyl phosphotriesters induced in the DNA of liver, kidney and lung after continuous ingestion of low concentrations of dimethylnitrosamine

Concentration of nitrosamine (p.p.m.)	Time from beginning of experiment (weeks)	Total dose ingested (mg per rat)	Phosphotriesters per 10 ⁶ nucleotides		
			Liver	Kidney	Lung
5	6	3.2	38.1(1.9)	−0.8(0.9)	4.2(0.7)
			31.9(1.6)		
	11	6.5	30.8(1.7)	−1.3(0.9)	4.8(1.2)
	17	8.2	4.8(1.7)	0.7(1.7)	6.9(1.3)
1	20	10.6	41.7(3.2)	0.7(1.9)	8.9(1.2)
	7	0.9	3.6(1.2)	−1.0(2.4)	2.4(1.5)
			3.8(0.9)		
	13	1.55	31.1(1.2)	0.9(1.2)	−2.6(1.3)
0.5	18	2.5	34.8(2.7)	3.5(1.4)	1.2(1.8)
	21	3.0	15.4(1.1)	0.1(1.5)	8.4(1.3)
	15	1.0	1.1(2.1)	−2.2(0.9)	—
	30	2.0	9.1(1.1)	1.0(1.3)	1.4(0.9)
0.1	38	2.6	5.5(1.5)	−0.3(1.0)	8.0(1.0)
			4.8(1.4)	5.0(1.5)	3.9(1.1)
			0.3(1.7)	3.0(1.4)	3.3(1.2)
	31	0.4	2.0(0.9)	1.0(1.1)	5.4(1.2)
	54	0.8	9.9(2.7)	1.4(1.3)	−1.2
			5.5(1.2)	2.6(1.7)	2.2(1.0)
			7.1(2.4)	0.2(2.6)	−3.5(2.1)
			17.0(1.1)	2.0(1.9)	1.1(1.0)

Groups of five female CB hooded rats were given drinking water containing various concentrations of the nitrosamine and the dosage estimated from the volumes consumed. Mean rates of consumption of the nitrosamine at concentrations of 0.1, 0.5, 1 and 5 p.p.m. were 2, 10, 20 and 75 μ g per rat per day, respectively. After different intervals a rat was killed, DNA was isolated from the three organs and the amounts of phosphotriesters present determined (see Table 1).

The results obtained from the experiments with diethylnitrosamine are presented in Table 3. It is again clear that, even at the low dose rates used, significant concentrations of ethyl phosphotriesters can be found in the DNA of all three tissues examined.

The positive result of the long-term feeding experiments described above has been to show that, after the course of a year during which the rats received only 2 μ g carcinogen d^{−1}, significant amounts of specific lesions could be detected in tissue DNA. These observations emphasise again that methyl and ethyl phosphotriesters are very stable in DNA and indicate that their accumulation in tissues could be monitored in relation to time of appearance of tumours.

Table 3 Concentration of ethyl phosphotriesters induced in DNA of rat liver, kidney and lung after continuous ingestion of diethylnitrosamine in the drinking water

Concentration of nitrosamine (p.p.m.)	Time from beginning of experiment (weeks)	Total dose ingested (mg per rat)	Phosphotriesters per 10 ⁶ nucleotides		
			Liver	Kidney	Lung
5	13	6.4	11.2(1.2)	−0.4(0.7)	3.3(1.3)
	19	12.7	31.6(2.9)	4.7(1.9)	5.2(1.4)
	34	20.2	21.1(1.3)	5.5(1.3)	2.1(1.2)
	42	25.9	42.5(1.8)*	4.7(1.3)	8.1(1.3)
1			14.9(1.6)*	8.4(1.4)	10.4(1.0)
	20	2.9	8.9(1.0)	2.8(1.6)	−0.8(2.2)
	35	4.8	16.6(0.9)	7.7(2.1)	5.2(1.5)
	43	6.2	8.4(0.5)	1.4(1.0)	−1.5(1.6)
0.1			6.4(1.3)	2.9(1.6)	5.9(1.5)
			8.7(0.8)	1.1(1.3)	1.2(1.5)
	29	0.3	9.2(2.0)	1.3(1.2)	4.9(1.6)
	52	0.7	13.6(2.2)	8.0(1.8)	−0.5(1.7)
			10.9(1.2)	4.5(1.4)	12.4(1.4)
			11.1(2.5)	4.6(1.6)	4.3(1.5)
			11.6(1.1)	14.3(1.3)	8.0(1.3)

*Gross tumours were found in the liver of these rats.

Details of the experiments are given in Table 2. Mean rates of consumption of nitrosamine at concentrations of 0.1, 1 and 5 p.p.m. were 2, 20 and 90 μ g per rat per day, respectively.

The proportion of phosphotriesters in the overall reaction products from DNA is known to depend on the nature of the carcinogen; with certain carcinogens, such as 7-bromomethylbenz[*a*]anthracene⁸ and the 9, 10-oxide of *cis*-or-*trans*-7, 8-dihydro-7, 8-dihydroxybenzo[*a*]pyrene^{9,10}, it seems to be relatively small. Conversely, there is, as yet, no record of a compound that reacts with the phosphates of nucleic acids which is not a carcinogen. These observations prompt questions about whether exposure of humans to the whole range of chemicals in their environment leads to the formation of significant amounts of phosphotriesters in their tissue DNA, and about whether the concentrations of such lesions can be related to cancer incidence. As the assay for phosphotriesters involves simply the determination of the number of chain breaks produced in DNA by alkaline hydrolysis, it should have a fairly general applicability, the nature of the chemical moiety reacting with the phosphate group being, in general, not important.

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The structure and replication of calicivirus RNA

RECENT work on the structure and replication of the caliciviruses—vesicular exanthema virus (VEV), San Miguel sea lion virus and feline calicivirus—has shown some interesting and unique features. First, the morphology is unusual and differs from that of other small unenveloped RNA viruses of eukaryotes^{1,2}. Second, the capsid structure is composed of 180 copies of a single polypeptide^{3,4}. Third, infection of cells with caliciviruses induces the synthesis of only three polypeptides, apparently produced independently of each other⁵. To gain a better understanding of this unusual pattern of protein synthesis and of the replication strategy, we have examined VEV RNA and the RNA species induced in cells infected with the virus.

VEV RNA, which is single-stranded and has a sedimentation coefficient of 37S, has been shown to be infective^{1,6} and thus can act as mRNA. We have now examined this RNA in more detail to determine whether it has any of the features common to eukaryotic mRNA molecules. The results of these experiments are summarised in Table 1. About 65% of the virus RNA bound to oligo(dT) cellulose in defined conditions⁷, indicating that the majority of the virus RNA molecules contain a poly(A) tract. The relatively low level of binding obtained can be compared with the results of Hruby and Roberts with encephalomyocarditis virus RNA⁸. In addition to the poly(A) tract at the 3' end it has been demonstrated that many mRNA molecules have an unusual nucleotide grouping at their 5' terminus⁹. For other plus-stranded virus RNAs, such as Sindbis virus RNA, this so-called 'cap' is of the form m⁷G(5')ppp(5')ApNp (ref. 10). Analysis of the products of a complete ribonuclease digest¹¹ of ³²P-labelled VEV RNA on DEAE-Sephadex in 7M urea failed to demonstrate the presence of a capped 5'-terminus. Using the same conditions we detected a capping group on ³²P-labelled Semliki Forest virus RNA. With VEV RNA most of the label was recovered in the mononucleotide peak (as would be expected) with very low and variable levels in the -3 and -4 regions, which is inconsistent with the presence of a capping group. A pancreatic digest of rRNA was co-run with the ³²P-labelled virus RNAs to characterise (by absorbance at 260 nm) the positions of the oligonucleotide peaks. It is interesting to note that the genomes of three picornaviruses, poliovirus, encephalomyocarditis virus and foot-and-mouth disease virus, which also act as mRNA, do not possess a cap^{12–15}. As with the picornaviruses^{15–18} we have found a protein covalently linked to the RNA of VEV¹⁹ but in VEV the molecular weight is approximately 10,000 compared with 4,000–5,000 found for the protein of the picornavirus RNAs.

Calicivirus RNA genomes thus possess certain features found in other plus-stranded virus RNAs with messenger properties, particularly the picornaviruses. The strategy of the caliciviruses, however, differs from that of the picornaviruses in that the three induced polypeptides are synthesised independently⁵. To correlate the possession of a single mRNA (MW 2.8 × 10⁶) with the independent synthesis of three polypeptides

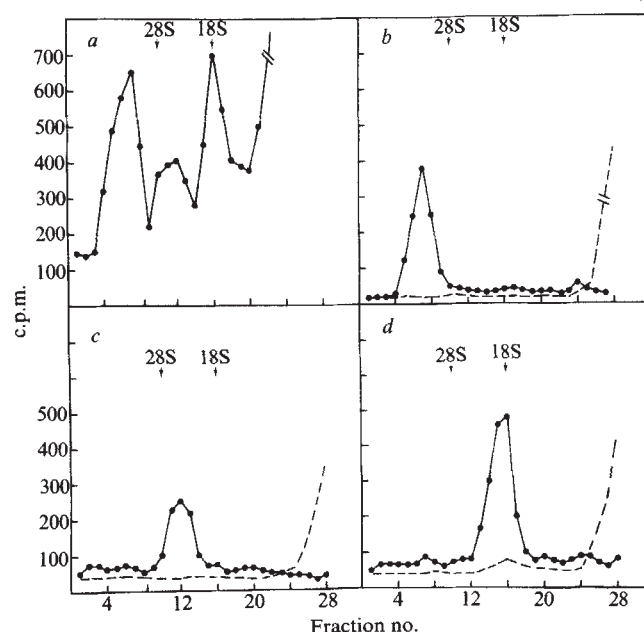
Table 1 Properties of VEV RNA and the induced RNA species found in cells infected with the virus

	RNase sensitive	% Binding to oligo (dT)-cellulose	% Hybridised to dsRNA
Virus 37S	+	65	ND
Induced 37S	+	30	60
Induced 22S	+	32	57
Induced 18S	+	40	60

Induced 37S, 22S and 18S RNAs were prepared and their RNase sensitivity determined as described in Fig. 1 legend. Virion RNA was prepared as described by Burroughs *et al.*²⁶. The presence of a poly(A) tract was ascertained by chromatography on oligo(dT)-cellulose columns⁷. Saturation hybridisation experiments with the isolated RNAs were carried out using unlabelled virus-specific dsRNA²². ds, Double-stranded; ND, not determined.

would seem to require the presence of three initiation sites on the mRNA. Internal initiation of this kind, however, has not been demonstrated for mRNA molecules in eukaryotic cells with the single exception of the flavivirus, Kunjin virus²⁰. An alternative strategy would be for each of the induced polypeptides to be translated from an individual mRNA. To distinguish between these possibilities, the RNA species induced in VEV-infected cells were analysed as described in the legend to Fig. 1. Three distinct species were obtained with sedimen-

Fig. 1 RNA species induced in IBRS-2 cells infected with VEV. Monolayers of IBRS-2 cells were infected with VEV (multiplicity of infection 100:1) for 30 min at 37°C and then incubated at 37°C in Eagle's medium (10 ml) containing actinomycin D (0.2 µg ml⁻¹). ³H-uridine (20 µCi ml⁻¹) was added 135 min after infection and the cells extracted with phenol-chloroform 195 min after infection. The extracted RNA was precipitated with ethanol, washed twice with ethanol/0.1M acetate (pH 5.0)–0.1% SDS (2:1) and analysed on 5–25% sucrose gradients in 0.1M acetate–0.1% SDS. a, Total extracted RNA. The fractions containing the 37S, 22S and 18S RNA were individually pooled and re-analysed with and without RNase treatment (50 µg pancreatic RNase and 50 units T₁ RNase per ml; 10 min at 37°C). ●—●, Without RNase; ---, RNase; b, induced 37S RNA; c, induced 22S RNA; d, induced 18S RNA.



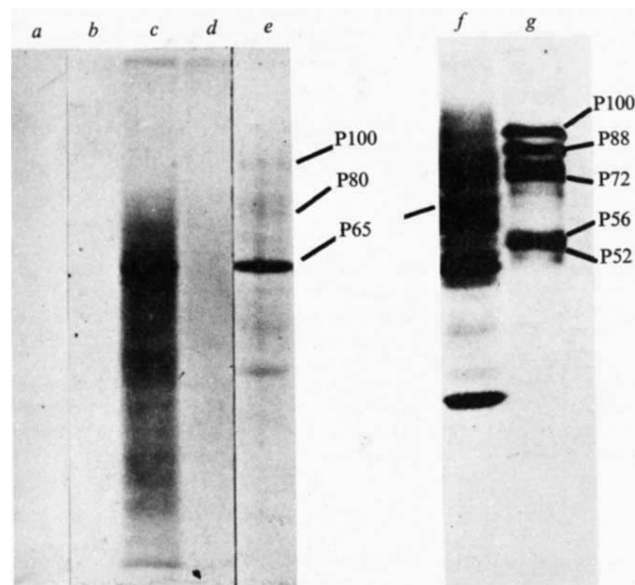


Fig. 2 Analysis of products synthesised in a reticulocyte cell-free system. The mRNA dependent reticulocyte cell-free system was prepared as described by Pelham and Jackson²⁵. 20 μ l Aliquots of this lysate were incubated at 30°C for 40 min with ³⁵S-methionine (2 μ Ci) and 1 μ g of the RNA under test. After samples were lysed (5 min at 100°C) with 50 volumes of lysis solution (0.77 g dithiothreitol; 1 g SDS; 4 ml 1 M Tris pH 6.8; 5 ml glycerol in 50 ml), the translation products were analysed on 10% polyacrylamide gels using the Laemmli system²⁷. Track a, control, no RNA added; b, induced 37S RNA; c, induced 22S RNA; d, induced 18S RNA; e, *in vivo* synthesised calicivirus-induced polypeptides; f, virus RNA; g, *in vivo* synthesised foot-and-mouth disease virus induced polypeptides to act as MW markers. Tracks a-e and f-g were run on separate gels.

tation coefficients of 37S, 22S and 18S. Ehresmann and Schaffer²¹ have also found 22S and 18S RNAs in addition to the virus RNA in cells infected with VEV. In our experiments the 37S and 22S RNAs were completely sensitive to pancreatic and T₁ RNase, showing that they are single-stranded. The 37S RNA is presumably the virus RNA and the complete RNase sensitivity of the 22S RNA eliminates the possibility that it is a replicative intermediate RNA. Although the 18S RNA was almost entirely RNase-sensitive there was a small quantity (<5%) of RNase resistant material in this fraction. This could be accounted for by the presence of a small amount of double-stranded replicative form (RF) RNA, which co-sediments with the single-stranded 18S RNA. In fact, later in the growth cycle (3–3½ h after infection) up to 25% of the RNA in the 18S fraction is resistant to RNase. This would be expected as RF is known to increase in quantity as infection proceeds in picornavirus-infected cells.

Chromatography on oligo(dT) cellulose columns showed that all three induced RNAs contained a poly(A) tract. As with the virus RNA the percentage of the RNAs bound to the oligo(dT) cellulose in defined conditions⁷ was rather low. For the induced 37S RNA it was 30%, for the induced 22S RNA 32% and for the induced 18S RNA 40% (Table 1). To demonstrate further the virus-specific nature of these three induced RNA species, saturation hybridisation experiments were performed with virus-specific double-stranded RNA isolated from infected cells as the source of complementary RNA²². For all three RNA species 60% of the labelled RNA added became RNase resistant (Table 1). Although it is difficult to decide whether only 60% of the induced RNA was homologous to the virus-specific double-stranded RNA or whether fragmented single-stranded RNA in the double-stranded RNA preparation was

responsible for this low value, the latter explanation is the more likely.

The presence of the 22S and 18S subgenomic RNAs in calicivirus-infected cells allows us to speculate on the strategy of virus protein synthesis. As the three polypeptides (P100, P80 and P65) are synthesised independently in calicivirus infected cells⁵ it is possible that the subgenomic RNAs code for these polypeptides. Such a strategy is not unique and has been demonstrated for tobacco mosaic virus²³ and Semliki Forest virus²⁴. Support for such a strategy comes from *in vitro* translation of the induced RNAs in a reticulocyte cell-free system. Nuclease treated reticulocyte lysates²⁵ containing ³⁵S-methionine were incubated with virus RNA or induced 37S, 22S and 18S RNA for 40 min at 30°C and the translation products analysed on polyacrylamide gel slabs. Figure 2 shows that the 37S and 18S RNAs did not seem to act as mRNAs in this system although the virus RNA was translated into polypeptides ranging in MW from 20 to 100 × 10³ including a polypeptide of MW 65 × 10³ which is possibly the capsid polypeptide (Fig. 2b, d, f). The induced 22S RNA, however, was translated almost exclusively into a single polypeptide which migrated to the same position as the *in vivo* synthesised polypeptide P65, MW 65 × 10³ (Fig. 2c, e).

It has been shown that in common with the picornavirus RNAs, calicivirus RNA possesses a poly(A) tract and a covalently bonded protein. However, their strategies of replication are quite different. Following infection with a calicivirus the RNA is transcribed into subgenomic 22S and 18S RNAs. These subgenomic RNAs are presumably the messengers for the virus-induced polypeptides. One of these, the 22S RNA, has been shown to code for a polypeptide that has the same MW as the coat polypeptide. We have been unable to demonstrate any messenger activity with the 37S and 18S induced RNAs in the reticulocyte system. However, this does not exclude the possibility that these RNAs have a messenger function because P100 and P80 are synthesised in such small amounts in infected cells that their synthesis in the *in vitro* system may be difficult to demonstrate.

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Diameter of haemoglobin S fibres in sickled cells

SICKLE-CELL disease is a manifestation of the assembly of the Hb S variant of human haemoglobin into fibres that alter erythrocyte morphology. Recent studies have focused on two distinct forms of the fibres—a 6-stranded form composed of stacked rings of six Hb S molecules^{1,2} and a more complex form³⁻⁵ which we have recently studied by three-dimensional image reconstruction⁶; this revealed a 14-stranded structure composed of 4 inner and 10 outer strands in an arrangement with hexagonal packing that produces an elliptical cross-section. A critical issue is the question of the predominant form of the fibres in sickled cells. In commenting on our paper describing the 14-stranded structure, Finch⁷ focused on the diameter of the fibres observed in sectioned sickled cells and the relationship of this parameter to the diameters observed in negatively stained preparations of isolated 6-stranded and 14-stranded fibres. He concluded that the 6-stranded fibres, with diameters of 16–18 nm (refs 1, 2), were more consistent in size with the diameter of fibres found in sectioned sickled cells. However, this conclusion was based on limited data for sectioned sickled cells and an apparent over-estimation of the diameter for the 14-stranded fibres. We have recently completed extensive measurements on the diameter of fibres in sectioned sickled cells based on centre-to-centre distances between fibres in bundles as well as measurements on the diameter of the 14-stranded fibres in negatively stained preparations that are corrected for flattening. Measurements have also been made on the centre-to-centre distance of fibres in bundles from stirred haemolysates^{5,8}. The results of these measurements are reported here and indicate an agreement in

Fig. 1 Electron micrograph of a section of an embedded sickled cell. Washed cells were deoxygenated for 2 h, fixed in deoxygenated 2% glutaraldehyde, stained in 1% OsO₄ for 1 h and embedded in Epon-Araldite. Before fixing, sickling was confirmed by examination with a light microscope. Positively stained sections of the embedded cells were examined with a Philips EM 301 electron microscope.

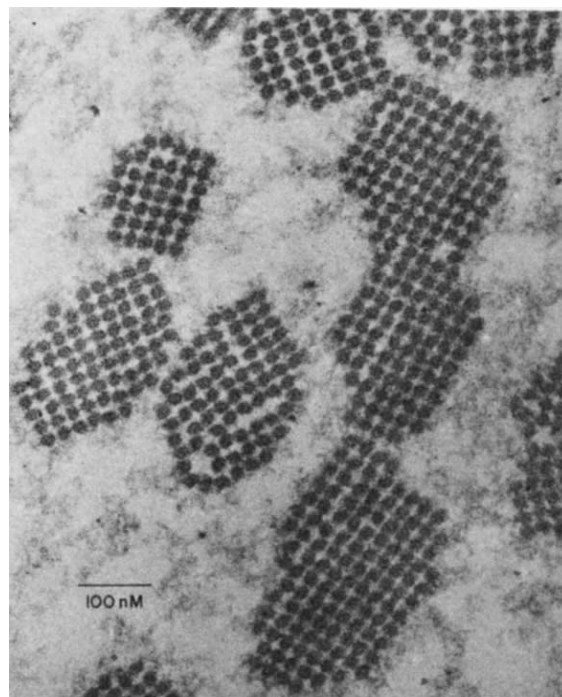
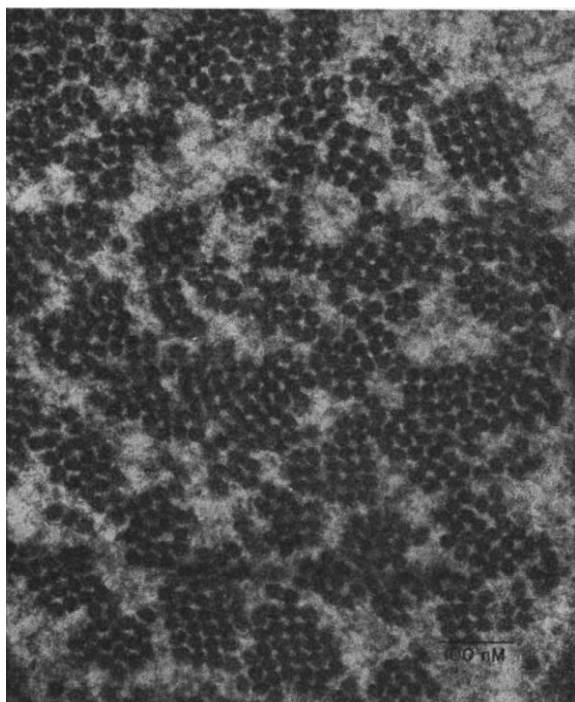


Fig. 2 Electron micrograph of a section of an embedded array of fibres from a stirred haemolysate. The fibres were prepared following the Pumphrey-Steinhardt method⁸ as described earlier⁵ and the material sectioned and examined as described in Fig. 1 legend.

the average diameter observed for the fibres in sickled cells and negatively stained 14-stranded fibres, with values in the range of 20–22 nm obtained in both cases. An average diameter in this range is also found for the fibres in the bundles from stirred haemolysates.

A positively stained section from a sickled cell obtained from a freshly withdrawn blood sample from a homozygous donor is shown in Fig. 1. Many clusters of Hb S fibres are apparent, including regions in approximately square or hexagonal arrays. The centre-to-centre distances for numerous adjacent pairs of fibres were measured and a value of 21.7 ± 1.1 nm was obtained. More highly ordered arrays of fibres can be obtained from stirred haemolysates⁵ and a section through such an array is presented in Fig. 2. In this case measurement of numerous centre-to-centre distances of adjacent fibres yielded an average value of 21.4 ± 0.9 nm. With the arrays from stirred haemolysates the order of the packing is sufficiently good that a conventional computer-based Fourier reconstruction can be carried out and the reconstructed image is presented in Fig. 3. The 14-stranded fibres were reported to have an elliptical cross-section on the basis of three-dimensional reconstructions⁶ and in the reconstruction shown in Fig. 3, the fibres can be seen to be aligned with their long elliptical cross-sectional axes along the sides of a square.

Finch suggested a maximum diameter of about 30 nm for the individual 14-stranded fibres structures⁷. However, our estimates (which also take into account recently measured flattening effects) are considerably lower. The data presented in Fig. 4 of ref. 6 indicate minimum and maximum radii of 7.7 ± 0.4 nm and 10.7 ± 0.5 nm, for centres of the outer strands. In order to obtain diameters, these numbers should be multiplied by two and have added the diameter of a single haemoglobin molecule (6 nm), giving minimum and maximum diameters of 21.4 ± 1.1 nm and 27.5 ± 1.4 nm. Our recent estimates from a series of tilted fibre micrographs indicate that there is appreciable flattening which would apply a 15% reduction to these numbers, yielding final minimum and maximum diameters of 18.2 ± 0.9 nm and 23.4 ± 1.2 nm or an average diameter of

20.8 ± 1.0 nm. This average diameter falls within the statistical range of 21.7 ± 1.1 nm estimated for sectioned sickle cells, so that the two numbers are concluded to be in agreement.

In summary, we conclude that there is no discrepancy between the diameters of the 14-stranded fibres recently described from negatively stained preparations⁶ and the diameters of the fibres observed in sectioned sickled cells, with both falling in the range 20–22 nm. The suggestion of a discrepancy⁷ was based on an over-estimate of the diameter of the 14-stranded fibres made before data on the flattening factor was available and an apparent underestimate of the diameter of fibres from sectioned cells in the older literature^{9–12}. This underestimate may be explained in part by measurements of diameters directly on single fibres in sectioned material for which the boundaries of the fibres would be difficult to specify precisely. In contrast, we have used centre-to-centre measurements of clustered arrays of fibres which should eliminate ambiguities due to variable stain penetration near the boundaries of the fibres. It should be noted that for sectioned

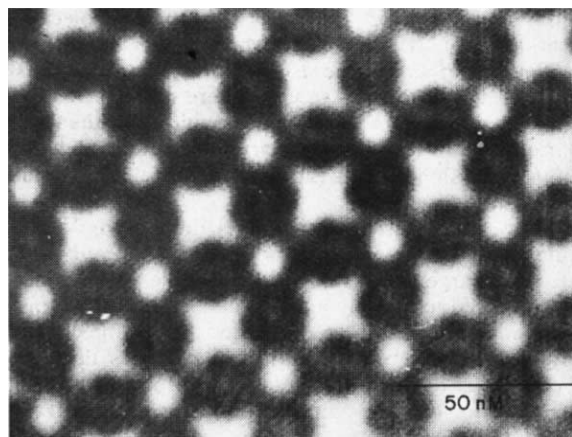


Fig. 3 A reconstructed image of a sectioned array of fibres. Electron micrographs of arrays prepared as described in Fig. 2 legend were transformed and reconstructed using the computer system and operations described previously⁶. The reconstructed image was displayed on a Tektronix graphics terminal and the image photographed for reproduction in this figure. The dark areas of the figure correspond to protein. In this section the long elliptical axes of the fibres happen to coincide with the sides of the square lattice; in other sections they do not, due to the helical nature of the structures.

gels Finch *et al.* reported square lattices with centre-to-centre distances of 23 nm (ref. 1). This value is close to our values for sectioned cells or sectioned bundles (21.7 ± 1 nm or 21.4 ± 0.9 nm). Thus we conclude that there is a general consistency between the average diameter we have determined for the 14-stranded fibres and the diameters observed for fibres from centre-to-centre measurements on sectioned sickled cells or haemolysates. The narrower, 17-nm fibres are evidently stabilised in certain conditions, but are not likely to be the predominant form in sickled cells on the basis of diameters that are too small. In addition, it is difficult to reconcile the 17-nm fibres with the fact that sectioned fibres inevitably appear solid (in agreement with the 14-stranded fibres which contain a 4-stranded core) while the 17-nm fibres are hollow, with a 6-nm wide space in the centre² that would be large enough to be seen in sectioned material. What remains unknown are the factors responsible for the stabilisation of the 6-stranded fibres in certain conditions and the stabilisation of the 14-stranded fibres in other conditions. In the work in our laboratory in the past 2 years with numerous preparations of fibres from lysed sickled cells, gelled haemolysates and stirred haemolysates involving many different homozygous sickle cell donors, we have not observed any six-stranded fibres. (Six-stranded fibres were seen occasionally in the previous two-year period, but

never at an abundance more than 1–2%.) It will evidently be an extremely difficult task to try to define the conditions which favour one form of the fibres over the other, but the bulk of the evidence we have gathered indicates that the 14-stranded structure is the predominant form of Hb S in sickled cells.

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Dimeric β -cyclodextrin complexes may mimic membrane diffusion transport

CYCLODEXTRINS have received considerable attention as models for biological macromolecules. Much of this interest has been stimulated by their suitability for serving as relatively low molecular weight models for enzymes which catalyse various hydrolytic reactions¹. Like enzymes, they form a complex with the substrate prior to the rate determining step of the reaction catalysed. We report here preliminary crystal structure analyses for isomorphous *n*-propanol and *p*-iodophenol complexes with β -cyclodextrin (β -CD) which we feel may provide interesting models for another aspect of biological chemistry, that of diffusion through a lipid bilayer membrane.

Crystallisation of the *n*-propanol complex, I, was effected by vapour diffusion of the alcohol into a solution of β -CD in 50% *n*-propanol/water. Crystals of the *p*-iodophenol complex, II, were prepared by slow evaporation of an approximately 30% methanol/water solution of β -CD containing a considerable excess of *p*-iodophenol. The common space group is P1 with two molecules of β -CD per unit cell. The lattice parameters (sequence *a*, *b*, *c*, α , β , γ , in Å and deg, respectively) for crystals at approximately -120°C are: 17.980(2), 15.424(2), 15.299(1), 103.0(1), 113.5(1) and 99.4(1) for I and 17.985(3), 15.352(3), 15.363(3), 102.8(1), 113.1(1) and 99.4(1) for II. High resolution X-ray diffraction data ($\text{MoK}\alpha$, $2\theta_{\text{max}} = 70^\circ$ for I and 55° for II) were collected for cooled crystals (approximately -120°C) with a Syntex P1 autodiffractometer equipped with a low temperature device (Syntex LT-1).

The initial crystal structure model was determined through the use of noncrystallographic symmetry in a manner that will be described in more detail elsewhere. Some difficulty has been encountered in definitively locating several of the carbon and oxygen atoms of the guest molecules in both structures. There is definable disorder in the orientation of one of the *p*-iodophenol guest molecules such that there is a fourth iodine atom position that had not been detected in the analysis of the Patterson map; we now estimate its occupancy to be approximately 25%.

Refinement of the structural model with a 1 Å resolution data set and isotropic temperature factors for all C and O atoms has resulted in $R = 12.5\%$ for I and 12.2% for II (iodine atoms refined anisotropically). At the present level of refinement, the chemical formula for the asymmetric unit of each structure is I: $C_{93}H_{164}O_{73} \cdot 24H_2O$ and II: $C_{102}H_{155}O_{73}I_3 \cdot 24H_2O$. As refinement of the structural models proceeds with anisotropic temperature factors and the higher resolution data, minor changes in composition may be detected, particularly with respect to the *n*-propanol content of I.

The structures of the inclusion complexes of I and II, including water oxygen atoms, are presented in stereoscopic projection² in Fig. 1. The two symmetry-independent β -CD molecules are coupled by intermolecular hydrogen bonds between secondary hydroxyl groups to form a dimer. In this respect, the structures are similar to that found for a 2:2 complex of β -CD with 2,5-diiodobenzoic acid³ and also to that shown by a β -CD *p*-nitroacetanilide complex⁴. Complexes I and II present different stoichiometry from either of these complexes and, as indicated, that of I is still subject to change; II displays clear 2:3 host:guest stoichiometry. Crystal structure analyses for α -CD complexes with *n*-propanol⁵ and *p*-iodophenol⁶ have also been reported. The latter is of particular interest because the orientation of the guest molecule differs radically from those in II. The guest molecule in the α -CD complex is aligned with its I→O vector nearly coincident with the local 6-fold symmetry axis of the host and is directed toward the side of the torus with the secondary hydroxyl groups (henceforth the secondary side). In II, these vectors for molecules A–C (Fig. 1), respectively, display acute angles of 36, 88 and 30° to the approximate local 7-fold symmetry axis; the vectors for molecules A and C are directed towards the side of the torus with the primary hydroxyl groups (primary side).

We find the structures of complexes I and II particularly interesting with respect to diffusion through a lipid bilayer membrane. The bilayer analogy is obvious but there are at least

two additional criteria that an appropriate model should fulfill. The dimer should exist in aqueous solution and there should be exchange between the contents of the dimer and the solution. The structures of I and II provide evidence that these criteria are met.

The thermodynamics of formation of CD inclusion complexes in aqueous solution have been discussed by Griffiths and Bender¹. In dilute aqueous solution, before complex formation, both substrate and host are fully hydrated. The mechanism of inclusion of a molecule with a hydrophilic substituent such as those in I and II very probably involves initial interaction (reciprocal solvolysis) between this functional group and a hydroxyl group of the CD molecule. The CD hydroxyl group can serve as a pivot point about which the guest molecule can swing into the hydrophobic cavity. This mechanism implies that the orientation of the guest molecule, with respect to the primary or secondary sides of the torus, is determined by which hydroxyl group it initially interacts with and by the size of the torus. For example, the α -CD torus is too small to allow a molecule of the size of *p*-iodophenol to enter through the primary side⁶, whereas a *n*-propanol molecule can apparently do so⁵. It is obvious from the structure of II that *p*-iodophenol can enter the β -CD torus from either side. Therefore, in dilute aqueous solution there should be a mixture of inclusion complexes with the hydrophilic substituent of the guest orientated towards the primary or secondary side of the host. If the inclusion process is purely statistical, this should be a 1:2 mixture, respectively.

The above mechanism assumes that the uncomplexed CD molecule is fully hydrated. The crystal structures of I and II indicate that a large number of water molecules (approximately 10^2) are required to hydrate a β -CD molecule. As the concentration of an aqueous solution increases (for example, by slow evaporation as in the crystallisation of II) or as the relative water content is decreased (increasing *n*-propanol content in the crystallisation of I) the number of water molecules available

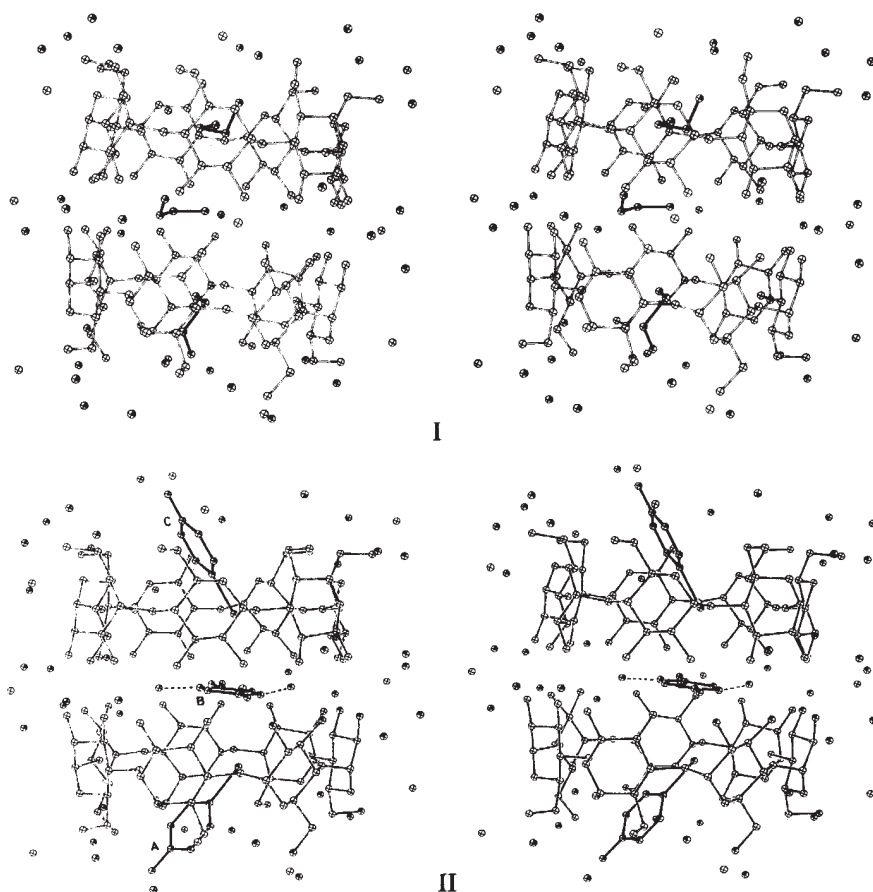


Fig. 1 Stereoscopic projections² of complexes I and II. The oxygen atoms of water of hydration molecules are drawn with shading. Oxygen atoms of neighbouring cyclodextrin molecules that may be regarded as part of the solvation of the 'dimer' are also included. Guest molecules are depicted with solid bonds, with the exception that the disorder in the central *p*-iodophenol molecule (B) is indicated with broken lines between the second iodine and oxygen atoms and the appropriate carbon atoms of the major ring system.

for hydration is decreased. The 'dimerisation' of the β -CD molecules provides a mechanism of reciprocal solvolytic compensation for inadequate *per capita* hydration. If crystallisation rapidly followed the formation of the dimer, a crystal structure analysis should reveal a complex in which the guest molecule is disordered such that its hydrophilic substituent is orientated toward either end of the β -CD torus. We suspect this to be the case for the 2,5-diiodobenzoic acid complex³ which was crystallised by cooling a saturated solution.

If the dimer is relatively stable in aqueous solution and complex formation proceeds as described above, there should be further exchange between the dimer and solution through the primary sides of the extended torus. In these conditions, the fraction of the complexes in which the hydrophilic functional group of the guest molecule is orientated towards the primary side of the host should approach one. Structures I and II indicate that such exchange has occurred.

The β -CD dimer also acts as a model for the concept that diffusion through a lipid bilayer membrane is an equilibrium process. The concentration of the solute in the hydrophobic cavity clearly reflects its concentration in aqueous solution. The 2,5-diiodobenzoic acid complex was prepared from a 1:1 mixture of components and maintains this stoichiometry, whereas complexes I and II were crystallised from solution with considerable excess of the guest compound. The composition of I and II may well represent the saturation state of the model membrane.

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Efficiency of protein production from yeast grown in liquor derived from anaerobically fermented tropical pasture

THE yeast *Candida ingens* grows well on both fermented pig wastes and rumen fluid and uses volatile fatty acids (VFA) C_2 – C_6 as a source of carbon and ammonia as a source of nitrogen^{1,2}. The *in vitro* fermentation of pasture also provides a suitable medium for the growth of *C. ingens*. This report provides evidence that pasture may be converted into edible protein in the form of yeast. The conversion is 16 times more efficient than in beef-producing animals. The method has potential for use in protein-deficient countries.

Minson and McLeod³ have described an *in vitro* technique for estimating the digestibility of large numbers of tropical pasture samples. In this technique 0.5 g samples of dried pasture are fermented anaerobically for 48 h with 40 ml buffer solution and 10 ml strained ovine rumen fluid. The liquor from

many of these batch fermentations was pooled and run into a tower fermenter⁴ which was inoculated with a culture of *C. ingens*. The liquor contained VFA at a concentration of 0.060 M. The fermenter had a total volume of 3.8 l and the volume of the medium was 3 l. There was a dilution rate of 0.04 h⁻¹ with an aeration rate, using air, of 600 ml min⁻¹. pH was maintained at 5 and the temperature of the medium was coincident with ambient at 21 °C. After steady state was reached, the effluent was continuously collected, the yeast separated by centrifuging, dried at 60 °C, weighed and analysed for proximate constituents and amino acid composition. The spent liquor contained 0.007 M total VFA.

Each 100 l of medium which contained the VFA produced by fermenting 1 kg pasture dry matter (DM) plus the VFA present in the rumen fluid inoculum, yielded 190 g of yeast dry matter. Of the VFA present in the medium 79% came from the fermented pasture so the net yield of yeast is 150 g dry matter for every 1 kg pasture DM fermented. The yeast contained 47% crude protein ($N \times 6.25$) giving a yield of 70 g crude protein from 1 kg pasture DM (Table 1). The nitrogen in the yeast represented 56% of the nitrogen obtained from the pasture and which went into solution as ammoniacal nitrogen. *C. ingens* fed to rats has been shown to have a potential as a fodder protein⁵.

For comparison the quantity of beef protein produced by a yearling steer was estimated using published nutrient requirement tables⁶. A steer of 350 kg liveweight gaining 0.5 kg daily would require 9.6 kg pasture DM of the type used in this study (49% dry matter digestibility). The 0.5 kg liveweight gain would contain 75 g crude protein; so for every kg pasture DM ingested, beef protein production would be 7.8 g. Of this protein about 58% is edible⁷, so the yield of edible beef protein from 1 kg pasture DM is 4.5 g. Thus yeast is 16 times more efficient at producing edible protein than beef cattle. If the cattle were gaining only 0.25 kg d⁻¹, which is quite common in the tropics, then yeast is 25 times more efficient than beef production.

The quantities of essential and non-essential amino acids produced in yeast were determined and compared with that of beef using published data on amino acid composition⁸ (Table 1). For all the essential amino acids yeast was at least nine times as efficient as beef production.

This study has shown that protein can be produced from pasture more efficiently by using yeast in a two-stage ferment-

Table 1 Yield of yeast and beef protein from 1 kg pasture DM

	Yeast (g)	Beef ⁸ (g)	Ratio yeast/beef
Total crude protein	70	7.8	9
Edible crude protein*	70	4.5	16
Essential amino acids			
Arginine	2.70	0.30	9
Histidine	1.20	0.13	9
Isoleucine	1.90	0.22	9
Leucine	4.00	0.37	11
Lysine	4.00	0.37	11
Methionine	1.20	0.10	12
Phenylalanine	2.70	0.18	15
Threonine	3.00	0.18	17
Valine	2.50	0.26	10
Non-essential amino acids			
Alanine	4.2	0.29	14
Aspartic acid	5.40	0.40	14
Cystine	0.90	0.07	13
Glutamic acid	7.50	0.64	12
Glycine	3.00	0.32	9
Proline	1.80	0.24	8
Serine	2.70	0.17	16
Tyrosine	4.20	0.14	30

* *C. ingens* has a nucleic acid determination of 9.19 g per 100 g crude protein². It is recommended that nucleic acid intake should not exceed 2 g per adult human per day (ref. 9).

tation system than by using beef cattle. The efficiency of yeast production is so high that it may prove to be a practical source of protein in some developing countries using locally grown pasture or the residue of other crops. Only relatively simple equipment would be required; operation would be simplified by the absence of any need to sterilise the medium, as the growth of undesirable organisms is inhibited by the high concentration of VFA and pH control. Further laboratory and pilot studies as well as feeding trials are now required to assess the full potential of yeast protein production from pasture.

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Spectral sensitivity of the red and yellow oil droplet fields of the pigeon (*Columba livia*)

THE function of the brightly coloured oil droplets situated within the cone receptors of the avian retina has long been the subject of debate^{1–4}. Among other hypotheses it has been suggested that they reduce glare and chromatic aberration¹, remove unwanted short-wavelength sensitivity², and have a role in colour vision^{3–5}. The transmission spectra of the droplets in the visible spectrum are typically those of short wavelength cut-off filters^{5,6–8}, due to the presence in high concentration of carotenoids of dietary origin^{9–11}. Recent evidence from microspectrophotometry suggests that the droplets filter the light reaching the visual pigment in the receptor's outer segment, resulting in the modification of the sensitivity functions of some of the individual receptors, from the broad band sensitivity of the visual pigment alone to a narrow band one^{12,13}. It is also known that marked differences occur in the proportions of the variously coloured droplets between species, and also between different parts of the retina of a given species^{4,14–16}. In the pigeon (*Columba livia*) the retina is divided into two distinct areas, known as the red and yellow fields, defined by their oil droplet populations. The red field is in the dorsal retina (frontal-ventral visual field), whereas the yellow field is in the ventral retina (dorsal visual field): the two fields differ in both the type and number of broad and narrow band receptors present, as well as in receptor density and the neural complexity of the retina^{13,17,18}. We report here clear differences in the photopic spectral sensitivity of the two fields, determined

behaviourally, and suggest that these reflect a difference in the colour vision mechanisms of the two areas.

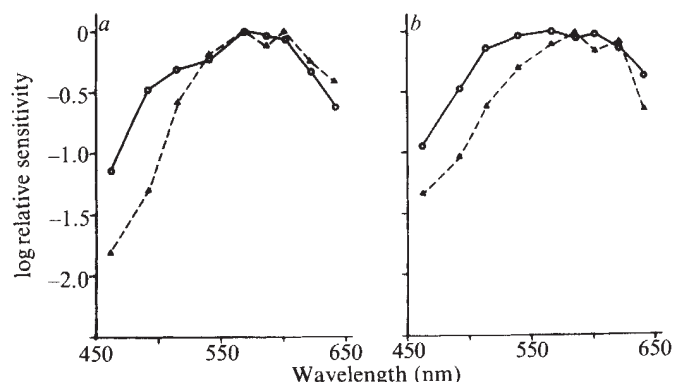
During behavioural testing the birds wore a translucent hood fixed rigidly to the head by means of two small bolts attached to the skull by dental acrylic. The hood was worn by the birds only during the experimental procedure. Vision along the line of the bill was unimpaired. Stimuli were presented to the red and yellow fields by means of fibre optic light guides attached to different points on the hood. The ends of the guides were polished and covered with a small piece of tracing paper to produce a uniformly illuminated patch (diameter 2 mm) of light positioned approximately 10 mm from the cornea, subtending a visual angle of approximately 12 degrees of arc. A two-choice discrete-trials simultaneous discrimination was used, in which subjects were trained to respond by pecking one response key when a stimulus light was present in either part of the visual field, and to peck the other in the absence of a stimulus light. Reinforcement was the presentation of grain. Stimuli were presented at the same wavelength but at different retinal loci on alternate days. Thresholds were determined by interpolating psychophysical functions (percentage correct responses against stimulus intensity) at the 50% correct level. Light entered the fibre optic at the focus of an optical system. An Hilger-Schwartz FT 17 thermopile placed at this focus was used to determine the relative energy available through the interference filters (Balzers, B40) and the neutral density filters (Kodak Wratten) *in situ*. A Shimadzu scanning spectrophotometer was used to determine spectral transmission of the filters. This apparatus and procedure will be described more fully elsewhere¹⁹.

Clear differences were found between the two spectral sensitivity functions for both subjects (Fig. 1). In each case the red field function is narrower than that of the yellow field, the yellow field is more sensitive at shorter wavelengths, and the two fields show similar sensitivity at wavelengths beyond about 600 nm.

There is a close similarity between the yellow field sensitivity function and that previously determined by Blough²⁰ for the pigeon in similar adaptation conditions. This suggests that in visual discrimination experiments involving brightness judgments, in which pigeons are free to examine stimuli with any part of their visual field, the yellow field (containing the fovea) is used.

Romeskie and Yager²¹ recorded in freely moving pigeons a spectral sensitivity function narrower than those found here, and they suggested that this narrow function represented the sensitivity of the red field. This interpretation, however, may be in error, as no consideration was taken of the possible selective adaptation effects produced by the high colour temperature adapting light used. Such adaptation effects could well have resulted in the relatively decreased sensitivity which they found below about 600 nm compared with Blough's results.

Fig. 1 Behaviourally determined spectral sensitivity functions of two pigeons (*a* and *b*) in the red (▲) and yellow (○) oil droplet fields. Sensitivity expressed according to an equal quantum intensity base.



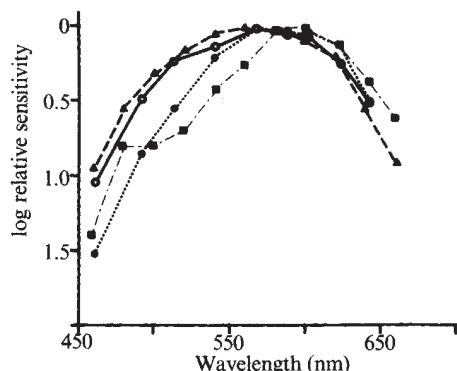


Fig. 2 Mean (two birds) spectral sensitivity functions of the red (●) and yellow (○) droplet fields compared with the modelled functions of the red (■) and yellow (▲) fields derived by Bowmaker¹³.

Good agreement is found between the mean yellow field sensitivity function of the two birds recorded here and that predicted by Bowmaker¹³ using a simple additive model, involving the summation of the spectral sensitivities of the individual cone types (visual pigment plus associated oil droplet) in the ratio of their numbers in the yellow field (Fig. 2). However, the agreement between the red field data and Bowmaker's similarly modelled red-field function is not so good.

The reason for this discrepancy is not clear. The size and position of the stimulus patch in the red field, close to the line of sight along the bill, makes it unlikely that the discrepancy is due to the measurement of a mixed response from both the red and yellow fields, which could have resulted in a broader function. Variability in droplet transmission spectra, however, could be a factor. The B-type droplets (approximately 30% of the red field droplets) are known to vary in transmission. Bowmaker¹³ when modelling his functions, selected the B-type droplets whose transmission spectra cut off at the longest wavelengths he recorded, thus producing the narrowest possible modelled red field function. It is possible that the red field function recorded here is from an area whose B-type droplets cut off at shorter wavelengths and this may account for the recorded function being broader than Bowmaker's modelled curve.

An alternative explanation for this discrepancy is that a simple additive model is inapplicable to this retinal area, and that some interactive model of receptor function of the kind thought to occur in photopic conditions in a variety of other animals²², is more appropriate. The red field is known to have a more complex synaptic organisation than the yellow field¹⁷, which may also be indicative of a difference in the neural interactions of the receptors underlying the spectral sensitivity of the two retinal areas.

Both fields contain cone receptors with both broad and narrow band sensitivity functions¹³. Although the broad bandwidth cones of each field have similar sensitivity functions, they vary markedly in their percentage abundance in the two fields. Both fields also differ markedly in their populations of narrow band receptors. The primary function of the narrow band cones is probably in the coding of colour information, the oil droplets performing at the level of the individual receptor a function similar to the 'spectral sharpening' of colour vision mechanisms in other vertebrate classes. We therefore propose that the differences in spectral sensitivity between the two fields which we have found reflect not only a difference in the sensitivity of the receptor populations, but also a difference in the neural mechanisms underlying photopic vision in these fields. The red field, by virtue of it containing more narrow band receptor types and having greater complexity of neural organisation, is probably capable of more subtle discriminations than the yellow field. Just what these differences are, and how they relate to ecological and behavioural variables, is clearly worthy of investigation.

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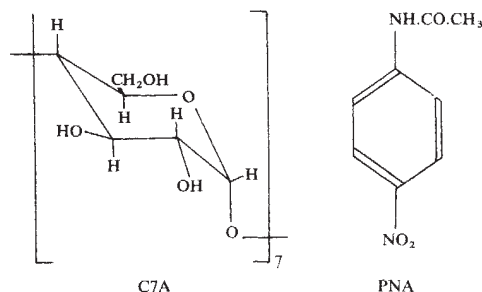
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Structure of the complex cycloheptaamylose-*p*-nitroacetanilide

THE cycloamyloses (CnA) are α -1, 4-linked cyclic oligomers of D-glucopyranose, which have attracted considerable attention as enzyme models¹. These doughnut-shaped molecules have the primary hydroxyl groups from the 6-position of the glucose residues at one side of the torus, and the secondary hydroxyl groups from the 2- and 3-positions at the other. On the inside of the cavity there is a ring of CH groups, a ring of glycosidic oxygens, and a further ring of CH groups, resulting in a hydrophobic ether-like interior. The ability of the cycloamyloses to form stable complexes with a variety of organic compounds by inclusion within the hydrophobic cavity has prompted their use as models for the active sites of enzymes. Of particular interest is the observation that they can accelerate the release of phenols from a variety of aryl esters² and of anilines from anilides³ by a reaction pathway similar to that observed for the hydrolytic enzyme α -chymotrypsin. Furthermore, a marked degree of substrate specificity is observed: thus, the cleavage of meta-substituted aryl acetates is accelerated more than that of their para-analogues². It has been suggested that this specificity is due to the closer positioning of the nucleophilic secondary hydroxyl group of the cycloamylose to the ester carbonyl in the meta complex than in the para complex². To test the validity of this hypothesis we have made an X-ray crystallographic study of a series of meta- and para-substituted acetanilides and report here on the 1:1 complex of C7A with *p*-nitroacetanilide (PNA).



C7A

PNA

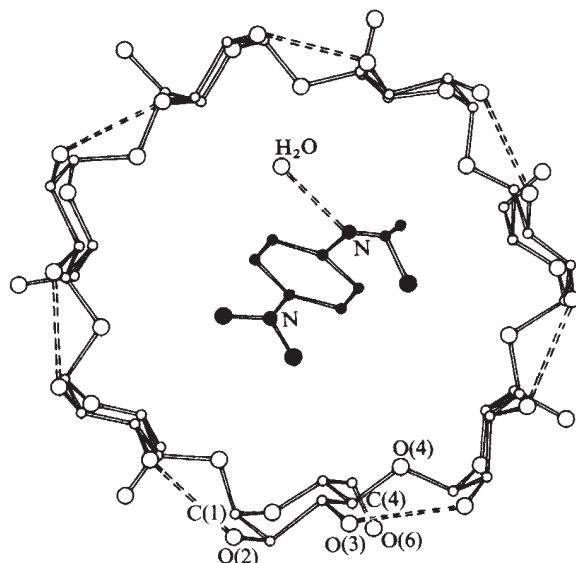


Fig. 1 One molecule of C7A, with its included molecule of PNA, projected along the approximate sevenfold axis. The larger circles represent oxygen atoms and the smaller carbon. The atoms of the PNA molecule are shaded. Dotted lines represent hydrogen bonds.

Stezowski⁴ has solved the structure of two other C7A inclusion complexes, those of *n*-propanol and *p*-iodophenol, and the structure of the 2,5-diiodobenzoic acid complex has also been reported⁵. Cyclohexaamylose inclusion complexes have been studied by Saenger⁶ and Harzuta⁷.

Crystals of C7A-PNA were obtained by slow cooling of 2:1 mixtures of C7A and PNA in water; for X-ray photography 'wet' crystals were sealed in Lindemann glass tubes. The crystals are triclinic, $(C_6H_{10}O_5)_7 \cdot C_8H_8N_2O_3 \cdot nH_2O$, where n is about 7, $a = 15.13 \text{ \AA}$, $b = 15.54 \text{ \AA}$, $c = 15.69 \text{ \AA}$, $\alpha = 88.65^\circ$, $\beta = 98.16^\circ$, $\gamma = 103.14^\circ$, space group $P1$, $Z = 2$. The crystals show pseudo-monoclinic diffraction symmetry (C_2), similar to several complexes reported by Hamilton and Steinrauf⁸, and suggesting that the two C7A molecules in the $P1$ cell are related by an approximate twofold axis.

Intensity data were recorded on Weissenberg films ($CuK\alpha$ radiation) and measured by the SRC Microdensitometer Service to give 5,500 independent reflections with maximum $\sin \theta/\lambda$ around 0.5. A model of the C7A molecule was constructed assuming seven-fold symmetry and using the geometry of individual glucose units as found in two C6A structures⁶. The Patterson function suggested that the sevenfold axes of the two C7A molecules in the unit cell are almost parallel (or antiparallel) and close to the c axis; it also gave an indication of the displacement between the molecules. The antiparallel arrangement, with approximate C_2 symmetry, was used, and structure factors were calculated for a series of models with each of the ring orientations (about the sevenfold axis) varied in 5° steps. Trial and error adjustment of the ring separation and tilt then led to an approximate solution with $R = 0.40$ for 500 reflections and $\sin \theta/\lambda < 0.24$.

Refinement involved many stages, mostly using the XRAY72 system of programs⁹; we used step refinement, later least-squares procedures, and at various stages Fourier and difference Fourier summations. The higher resolution data were gradually introduced, and bond lengths and angles were maintained close to standard values by the program 'Modelfit'¹⁰. One molecule of PNA was found in the difference Fourier calculated with 2,900 reflections ($E > 0.5$ and $\sin \theta/\lambda < 0.4$) at $R = 0.25$; the second PNA molecule appeared in a subsequent difference Fourier calculated with the full data set; water molecules were also found.

At present, $R = 0.17$ for 5,500 observed reflections (one isotropic vibration parameter for all atoms). The PNA molecules have been assigned a site occupancy of 0.75 on the basis of the peak heights in the electron density maps, but it is possible that the sites are fully occupied by atoms with higher vibration parameters. In all, 194 non-hydrogen atoms have been located, 77 in each C7A, 13 in each PNA, and 14 water molecules. Figures 1-3, prepared with the program Pluto¹¹, illustrate the structure. Formally estimated standard deviations of atom positions are about $0.05 \text{ \AA}$ in the C7A molecules. Further refinement is planned.

All glucose units have the C1 chair conformation and dimensions which do not differ significantly from standard values. Each C7A molecule has the expected torus shape with the PNA molecule in the cavity. The PNA molecule is tilted so that its N-N axis makes an angle of 30° with the sevenfold axis

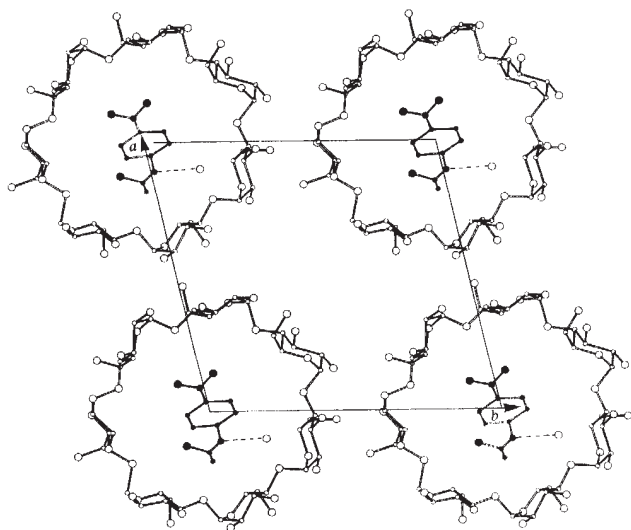


Fig. 2 c -Axis projection of half the structure. The other C7A molecules lie immediately above those shown here.

of the C7A ring (see Fig. 3); in this way the oxygen atoms of the nitro group are approximately in the plane of the seven primary hydroxyl groups (O(6)'s) and the acetyl group is level with the secondary hydroxyl groups (O(2)'s and O(3)'s). Each anilide nitrogen makes a hydrogen bond with a water molecule within the cavity. The C7A molecules are only slightly distorted from sevenfold symmetry. The torsion angles ϕ and ϕ' , ($\text{O}(4)_n\text{-C}(1)_n\text{-O}(4)_{n+1}\text{-C}(4)_{n+1}$ and $\text{C}(1)_n\text{-O}(4)_{n+1}\text{-C}(4)_{n+1}\text{-O}(4)_{n+2}$, n is the glucose residue number) have mean values of 173 and -175° with variations around each C7A molecule of $\pm 10^\circ$. In cyclohexaamyloses⁶ the mean values of ϕ and ϕ' are 166 and -169° with similar variations.

There are no hydrogen bonds or other contacts $< 3.2 \text{ \AA}$ between the C7A molecule and the PNA in its cavity; one of the associated water molecules is very weakly hydrogen bonded ($3.2 \text{ \AA}$) with a glucose O(3). O(3) of each glucose unit is hydrogen bonded to O(2) of its neighbour; the mean distance is $2.79 \text{ \AA}$, the range $2.6\text{--}3.0 \text{ \AA}$. Very similar hydrogen bonding occurs in cyclohexaamyloses⁶.

Pairs of C7A molecules associate face to face with hydrogen bonds between their O(3)'s (mean distance 2.80 , range 2.7--

$3.0 \text{ \AA}$). The closest approach between PNA molecules is $3.8\text{--}4 \text{ \AA}$ (between acetyl groups; if the PNA molecules were not tilted the acetyl groups would be impossibly close). These face-to-face pairs of C7A molecules are stacked along the c axis (Fig. 3) to produce the characteristic 'channels'.⁸ One pair of primary hydroxyl groups (O(6)'s) of neighbouring molecules within a stack is directly linked by hydrogen bonds, and there are additional links through water molecules. Such head-to-head stacking is found in all other C7A complexes^{4,5} but only in one C6A complex⁶. Neighbouring columns are close-packed (Fig. 2) and there are several hydrogen-bonded links between hydroxyl groups of neighbouring columns and others through water molecules. O(4) and O(5) atoms are not involved in any hydrogen bonds $< 3.0 \text{ \AA}$.

The PNA molecule has been found, as expected, in the cavity of the C7A. There are no specific interactions apart from very weak hydrogen bonding through a water molecule, so we conclude that van der Waals forces are responsible for holding the PNA molecule in the cavity. The tilt (Fig. 4) was initially a surprise, but it allows the PNA molecule to occupy most of the available space in the C7A cavity while keeping the polar nitro and amide groups close to the hydroxyl groups of C7A and the nonpolar benzene ring in contact mainly with CH groups. If a molecule of *m*-nitroacetanilide were similarly held in the C7A cavity its acetyl group would probably approach the secondary hydroxyl groups; however, conclusions about this must await the structure determination of the *m*-nitroacetanilide complex, which we are pursuing.

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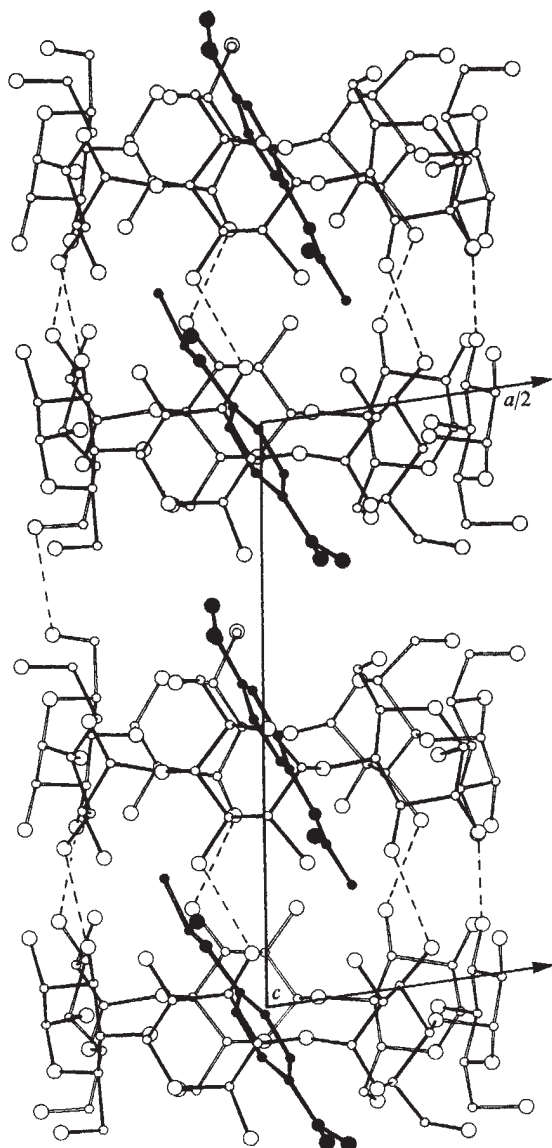
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Fig. 3 b -Axis projection of one column of C7A molecules. Seven hydrogen bonds link the O(3)'s of neighbouring molecules up the stack.



matters arising

Dissociative recombination and meteor trains

SODIUM D-LINE emission arising from the cyclic process



which was originally discussed¹ in connection with trains produced by 'fireball' meteors (brighter than visual magnitude -5) could, by virtue of an upward revision of the coefficient k_{Na} by three orders of magnitude², be significant for fainter visual meteors (down to zero magnitude)^{3,4}. The observed brightness of a train in sodium light is expected to remain essentially constant until it is resolved by the observing instrument. Here we draw attention to dissociative recombination of metal monoxide ions as a source of train luminosity⁵. The ions are produced by the process



(M = Mg, Ca, Fe)

and the subsequent emission begins to decay immediately in quasi-exponential fashion. Emission may also be associated with the mutual neutralisation of M^+ ions with atmospheric negative ions⁶, but is probably significantly weaker⁵.

The rate-controlling process in both equation (1) and equation (2) is the oxidation by ozone, and the initial ratio of photon rates (per unit length of train) may be shown to be

$$(\text{Recombination emission/Sodium emission}) = (p_+ \beta_{\text{M}} \alpha_{\text{M}} k_+ / p_{\text{Na}} \alpha_{\text{Na}} k_{\text{Na}}) \quad (3)$$

where α is the linear density of ablated atoms indicated by the subscript, p_+ and p_{Na} are the appropriate mean photon yields per initial oxidation and β_{M} is the mean probability that an ablated M atom will be ionised. The relative contributions of these two components to visual or photographic data will depend largely on the spectral characteristics of the recombination emission. A recent examination of the role of unstable metal monoxide states in the dissociative recombination process has enabled me to estimate relative excitation cross sections for various possible dissociation products and hence obtain approximate relative multiplet strengths. The main contributors are

found to be, using the identifying numbers of Moore⁷, Mg I 1, 2, Ca I 1, 2, Fe I 1, 2, 3, 4, 5 and, to a lesser extent, other multiplets of Fe. The weighted mean wavelength of this radiation, allowing for the relative abundances of ion species in the train, is $\lambda \approx 430$ nm. If we take account of the response of the dark-adapted eye (scotopic condition) we find $\lambda_{\text{eye}} \approx 480$ nm (blue). There are also strong lines in the green and red.

From adopted elemental abundances for typical stony meteoroids we take $\alpha_{\text{M}}/\alpha_{\text{Na}} = 50$. The author's analysis gives $p_+ \approx 0.25$ and for p_{Na} we adopt 0.4 (ref. 4). β_{M} increases rapidly with increasing meteor speed, reaching ~ 0.06 at 60 km s^{-1} (after Sida⁸). With $k_{\text{Na}} = 3.3 \times 10^{-16} \text{ m}^3 \text{ s}^{-1}$ (ref. 2) and $k_+ = 2 \times 10^{-16} \text{ m}^3 \text{ s}^{-1}$ (a mean value⁹) we find the ratio in equation (3) to be small for slow meteors, increasing to unity for a speed of $\sim 60 \text{ km s}^{-1}$. Allowing for the relative insensitivity of the dark-adapted eye at the sodium wavelength, the speed for which the two components are equally visible is $\sim 45 \text{ km s}^{-1}$. Blue recombination multiplets are thus expected to dominate at higher meteor speeds, especially for the eye.

The emergence of recombination as a significant source of emission is consistent with the suggestion^{3,10} that train luminosity associated with the fainter visual meteors is likely to be ionisation-linked. If no other processes are operative such trains might exhibit any hue from yellow through white to the complementary blue, and change with time depending on the decay characteristics of the two components. The fact that decays recorded photographically over the years generally agree with the predicted behaviour of the recombination component, can presumably be ascribed in part to the use of blue-sensitive film in much meteor work. Unfortunately, data against which these conclusions could be checked are scarce. Visual observations of faint short-lived trains tend to be unreliable and spectra, or even colour photographs, are difficult to obtain.

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BAGGALEY REPLIES—The oxide-reduction sodium cycle process seems to be incapable¹ of yielding observable meteor train emission for meteors fainter than visual magnitude about $-3\frac{1}{2}$ (velocity 45 km s^{-1}), and a catalytic cycle is incapable of exciting levels responsible for allowed transitions in meteoric atoms². As some of the emission characteristics expected from the dissociative recombination of metal oxide ions are compatible with observations, the process seems to be an attractive candidate for train luminosity³.

Although Poole suggests³ that no useful meteor train spectral data exist, it is interesting that experienced observers in the last century using specially designed prismatic instruments did identify^{4,5} spectrum line features (particularly Na I (ref. 1) and Mg I (ref. 2) in agreement with dissociative recombination) as well as a continuum in enduring trains. However, the conclusion³ that emission in the blue ($\sim 4,300 \text{ \AA}$) would dominate (due to Fe multiplets) contrasts with the photographic experience of early observers⁶ that meteor trains failed to register on ortho films; and indeed the few published records of trains were obtained using film emulsions⁶⁻⁸ with response extended to about $6,500 \text{ \AA}$. Further, the only recent spectroscopic information is provided by Whipple's grating observation⁶ of a train which revealed line emission in the red together with an orange-red continuum.

In dissociative recombination no rules are available to determine the proportion of reaction energy released as translational energy of the products. Although it is generally expected that, provided suitable states are available, all reaction energy emerges as atom excitation, the estimated³ luminous efficiency (p_+) awaits experimental confirmation.

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reviews

Zoological collections under fire

R. J. Wheater

The Last Great Wild Beast Show. By Bill Jordan and Stefan Ormrod. Pp. 271 (Constable: London, 1978.) £6.50.

THE authors of this book have had considerable practical experience with various aspects of animal management. Their book traces the development of animal collections from the Persian 'Paradise' to the modern specialist collection.

The roles of the zoological collection in research, education, conservation and recreation are identified and the failure of those responsible for such collections to pursue these roles are noted. The capture of the animal in the wild and its progress through local quarantine, transportation by various means to the collection through dealers is examined and the failures highlighted.

The authors then examine the hazards that face the animal that has survived thus far when it arrives in the collection. Examples of poor accommodation, badly designed and therefore stressful exhibit areas, unsatisfactory animal husbandry both with regard to diet and keeper care, are illustrated in a whole series of incidents where things went badly wrong.

The final chapter sets out to justify the retention and development of specialist collections; the best of the existing ones are described; and the abandonment of the mainstream zoo and safari park is recommended.

One of the photographs illustrating this book shows a large collection of Elephant lower jaws collected in the Tsavo National Park in Kenya from the skeletons of animals which died within the Park from natural and unnatural causes, jaws gathered together to obtain age data for scientific investigation into the population dynamics of this species. The authors' caption reads "The fate of the Elephant? Several thousand skulls confiscated from poachers in Tsavo National Park, Kenya". At least the location and the species were accurately identified. The caption to this one picture epitomises all that is wrong with this book—the lack of accuracy, important omissions, a fitting of facts or half-truths to suit the authors' preconceived ideas.

All is by no means perfect in the

way animals are captured and transported, but rules and codes of practice have been instituted by those responsible. The design and construction of animal accommodation and exhibits does not always fulfil the need. How can those responsible be expected to do better for animals than architects and planners can do for the human animal? Those developments which the authors applaud have evolved from a better understanding of the needs, in the same way that something better will, it is hoped, evolve from the era of the high-rise flat (tall apartment building).

All animal collections have a potential for the development of serious education programmes and regrettably many have failed to respond to the challenge. There are those who, at no small cost to themselves, have created very professional, fully utilised and highly respected programmes in conservation and environmental education. These efforts are not considered worthy

of mention.

The recognition by some zoos of failures and shortcomings in many aspects of animal husbandry and exhibit design led to the publication in 1959 of the International Zoo Yearbook, the establishment of the Federation of Zoological Gardens and, later, the National Zoological Association. These, coupled with symposia and meetings on a wide field of subjects and mutual problems, have gone a long way to alleviating many of the failures and have created a far greater degree of co-operation and coordination of effort. These positive developments receive scant attention from the authors.

Readers of this work should have no difficulty in applying a higher degree of objectivity towards the subject than that achieved by the authors. □

R. J. Wheater is Director of the Royal Zoological Society of Scotland, Edinburgh, UK.

Endocrinology annual

The Year in Endocrinology, 1977. Edited by S. H. Ingbar. Pp. 399. (Plenum: New York and London, 1978.)

As the editor states in his preface, with the publication of this second volume in the series the book becomes truly annual. The event is in keeping with its declared objective "to assist interested members of the biomedical community in coping" with "the continuing, incredibly rapid, growth of knowledge in all aspects of endocrinology".

Of the 11 chapters which the book contains, one, Autoimmunity in Endocrine Disease, is defined by the editor as non-recurring, and a second (Vasopressin and Water Metabolism) as biennial. The remaining nine chapters are therefore to be classified as annual and are entitled Hypothalamus, Anterior Pituitary, The Thyroid, Parathyroid Hormone and Calcitonin, The Adrenal Cortex, Aldosterone and the Renin-Angiotensin System, The Ovary, The Testis and, finally, The Sympathoadrenal System.

The individual authors, most of whom are endocrine physicians, have

reputedly signed on for a five-year term. They have clearly been given rather loose instructions for the design and preparation of their several chapters, and the overall bias of the accumulated text is towards the presentation and discussion of clinical data. This suggests that the "interested members of the biomedical community" for whom the series is intended were thought of mainly as clinicians.

The book starts with Dorothy Krieger's excellent chapter on the hypothalamus, which is surprisingly up to date considering the rapidly accelerating pace at which new knowledge in this area appears in print. New studies on the pituitary portal system are reported and these are illustrated by diagrams and scanning electron micrographs; these last would perhaps have been better shown in colour translation rather than black and white. Retrograde blood flow from pituitary to brain is envisaged as an anatomical reality.

William Daughaday presents, as always, a succinct functional outline of the anterior pituitary, each division starting with basic knowledge and proceeding rapidly to physiology, phar-

macology and pathology. His chapter ends with a long discussion on somatomedins.

The thyroid gland is dealt with by Woeber and Braverman who use the same format as in the earlier volume. Considerable emphasis is placed on synthesis and secretion of T3 and T4, and on the mechanism of their action. Calcium homostasis is very competently discussed by a father-daughter team (Kleeman and Kleeman), mainly with reference to parathyroid. This chapter proceeds to some speculations about the mechanism by which parathyroid cells respond, usually, to low calcium levels and the reasons for their failure to store their product in endocrine type granules.

John Gwynne and Robert Ney together survey the adrenal cortex, beginning with a discussion on "big" ACTH and ending with sections on Cushing's disease and Nelson's syndrome. Edward Biglieri outlines the renin-angiotensin system with a most useful diagram or flow chart as a starter, ending with a survey of various aldosteronisms. Gary L. Robertson then describes the physiology of vasopressin, with reference to osmoregulation, baroregulation, hormone regulation and other stimuli. He considers also diabetes insipidus and inappropriate antidiuresis.

The various amenorrhoeas are the province of Lipsett and Ross, whose survey includes the post-pill variety of that symptom and also the HCG-connected disorders. Next comes Ferman's exposition of the testis which begins with the problem of sex differentiation in the embryo, embracing the Mullerian regression factor and the dynamics of pituitary gonadotropin

function. Proceeding by way of childhood and puberty he presents a thorough discussion of the regulation of the hypothalamic-pituitary axis by testicular products including the long-postulated FSH-releasing inhibitor "inhibin".

Lewis Landsberg deals with the sympathoadrenal system and provides simplified schemes for the regulation of central sympathetic outflow and for the effects of catecholamines on (the integration of) peptide hormone secretion. Several sections are devoted to the role of the system in essential hypertension, and the final lucid chapter, by Silverberg and Volpé, presents a general model of autoimmune disease as it affects the gonads and the thyroid, adrenal, parathyroid and pituitary glands.

The essays which comprise this volume are of uniformly high standard in terms of style, presentation and content of factual information. This is an unusual feature in multiauthor handouts, for which I am by no means an enthusiast. But works of this calibre almost effect a conversion. There is inevitably a considerable degree of overlap in the subjects dealt with in the various chapters, but considering the essential unity, at least of the peptide hormone-producing cell systems, this is hardly surprising.

This second volume of *The Year in Endocrinology* contains something for every clinician with even a trace of endocrine feeling (surely 100% of those on the register) and something also for most like-minded biologists.

A. G. E. Pearse

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One view of the brain's energy metabolism

Brain Energy Metabolism. By B. K. Siesjö. Pp.606. (Wiley: Chichester, New York, Brisbane and Toronto, 1978.) £17.50.

WRITERS have recently furnished neurochemistry with many volumes of multi-author publications but with few monographs in which an author explored his chosen subject critically, systematically and at length. Dr Siesjö's book is greatly to be welcomed for being such a review and for its detailed compilation of data. No other volume carries such a wealth of information on the major energy-yielding processes of the brain, and in appraisal of cerebral energy status, in terms mainly of

phosphocreatine, adenosine triphosphate, less phosphorylated nucleotides, and reactions which interconvert those compounds.

The book commences with data, definitions and principles concerning metabolism, free energy and work. Thereafter, it is concerned mainly with the brain of a few mammalian species, including man; data on the brain of other animals is admittedly less complete, though they had and still have significance in pioneering and comparative studies of the book's main theme. Of particular note are those findings concerning cerebral blood supply and substrate and oxygen utilisation, specifically in man. Regional studies of cerebral blood flow and respiration in the brain are especially impressive for the correlations which they are now offering with localised changes in cerebral functioning, as examined by concomitant psychological testing in

man. These findings may merit description at a greater length than that given by Dr Siesjö, especially as results continue to be accumulated from computer-assisted interpretation of data obtained by scanning methods.

The preponderantly mitochondrial source of the metabolically derived energy of the brain is described, though it is not always clear from the text which investigations of those quoted have been carried out with mitochondrial preparations from the brain. Information on cerebral systems intermediate in complexity between subcellular entities and the whole brain is not well presented, and is occasionally incorrect. No description is given of the isolated cerebral subsystems, prepared and maintained with due consideration of metabolic supplies and anatomical integrity, in which both energy metabolism and the concomitant electrophysiological activity can be observed. These subsystems, and indeed simple tissue slices from several cerebral regions, frequently display spontaneous action potentials and thus raise the question of what endogenous cerebral processes may correspond to the endogenous generation of electrical activity in these undoubtedly deafferented preparations.

The book's main trend, however, is away from questions of the brain as an initiator of energy-use. Most of the information quoted on cerebral energy metabolism is derived from changes imposed by external variables: induced seizures, hypoglycaemia, ischaemia, anaesthesia, sedation and the increase or decrease in carbon dioxide or in temperature. Judging the brain from the point of view of these variables understandably contributes to a particular outlook on its activities, in which response to imposed states dominates over recognition of it as a source of thought and self expression.

The book's format is not consistently helpful to the reader. Units are well systematised in a preface but abbreviations are not listed, which is particularly unfortunate in a multidisciplinary subject. Three-letter abbreviations are ubiquitous; a summary paragraph of a few lines includes RAS, CMR and CBF, which do not have index entries; RAS has been specified as reticular activating system some six pages previously but even under its full name does not appear in the index. Running titles or chapter numbers at the heads of pages are lacking. Tables and Figures are well presented and the bibliography is extensive.

Henry McIlwain

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Compelling curiosity of naturalists

A World of Naturalists. By J. Kastner. Pp.350. (John Murray: London, 1978.) £7.95.

ONE reviewer of J. Bigelow's *American Medical Botany* (1817-20) expressed "a concern that too few American botanists were actively studying the plant life of their country . . . While we ourselves were devoted to the more profitable employment of commerce and agriculture, a Catesby, a Kalm, a Michaux or a Pursh was reaping a harvest of fame on our soil, and teaching us to admire the grandeur of our majestic forests and luxuriant savannahs." Although there were native-born American naturalists like the Bartrams, Mr Kastner's book is largely a chronicle of the achievements of those immigrant naturalists who confounded the parochial view of the French naturalist, Comte de Buffon, that the fauna and flora of the New World were inferior to those of the Old World.

Starting with John Banister, a young clergyman who went to Virginia in 1678 resolved to write an account of its natural history, Mr Kastner follows

the progress of American natural history through the activities of such men as Peter Kalm (one of Linnaeus's favourite pupils); and André Michaux and Thomas Nuttall, whose floras of North America remained standard works for many years. The first native American botanist, John Bartram, whose garden in Philadelphia is now regarded as the first true botanical garden in North America, collected plants for European gardens and was made the King's botanist in America. The *Travels through North and South Carolina* [etc] (1791) by his son William, likewise a plant-collector, was the source of much of Coleridge's poetic imagery.

The generous extracts Mr Kastner provides from journals, letters and published works convey vividly the wonder and astonishment evoked by the discovery of so many new plants and animals. The determination of these early naturalists to bring back specimens of what they saw was not without considerable danger to themselves. John Lawson, who like Banister before him had intended to write a natural history of Virginia, suffered a lingering death at the hands of hostile Indians who ignited small splinters of wood stuck all over his body.

The story of American natural history as it emerges from the pages of Mr Kastner's book is also the story of the discovery and exploration of the North American continent.

What all naturalists have in common is a compelling curiosity. Thomas Nuttall earned for himself the nickname 'Old Curious' and Constantine Rafinesque, oblivious of the ridicule of his contemporaries, reported the results of his insatiable curiosity in a flood of papers that are only now being fully appreciated by scientists. It was Rafinesque who said that although "a life of travels and exertions has its pleasures and pains . . . you feel an exultation, you are a conqueror, you have made a conquest over nature, you are going to add a new object, or a page to science".

With such remarkable and idiosyncratic men as those in his subject, Mr Kastner could not fail to write an absorbing book. His bibliography testifies to the extent of his research, and the illustrations, including a number in colour, are well chosen.

Ray Desmond

Ray Desmond is Deputy-Keeper at the India Office Library and Records and a Past-President of the Society for the Bibliography of Natural History.

Tumour immunology

Immunological Aspects of Cancer. Edited by J. E. Castro. Pp. 477. (MTP Press: Lancaster, UK, 1978.) £14.75.

TUMOUR immunology has reached another stage in its tortuous evolution where doubts are being voiced as to the wisdom of its rapid expansion in recent years. It is perhaps unfortunate, therefore, that this new multi-author book should appear just at the time when a reappraisal is being made of the many immunological approaches to cancer. In consequence, the reader will find little about the latest 'popular developments', such as the role of natural killer cells in immunosurveillance. One would, therefore, expect a critical account of the past achievements in tumour immunology, so that the reader can be led into the subject and decide whether it has any appeal. This objective is only partially fulfilled and it is unfortunate that a number of the chapters show the author's own prejudices all too readily.

The basic concepts of tumour immunology as defined in animal systems are reviewed in a concise

manner. Michael Moore's summary of neoantigens in animal tumours is of particular value, even though spontaneous tumours are not given the attention they deserve. The role of effector cells (T lymphocytes and macrophages) in tumour rejection and the effects of humoral factors modifying these responses are also well presented. At least here the authors point out many of the shortcomings of the approach being used to study immunity to cancer. The critical approach adopted by Suzanne Eccles in reviewing the role of macrophages is particularly appealing and Peter Beverley's account of T lymphocytes is excellent, even though too concise for the non-specialist.

It is when one moves to considerations of the role of immunological factors in human cancer that judgement becomes clouded by optimism. For example, the brief review of *in vitro* methods for detecting human tumour-associated antigens leaves one with the impression that none of the approaches are adequately developed to command widespread acceptance. Yet the reader learns later that these techniques can be used for evaluating immune reactions in cancer or even for immunodiagnosis. The same dichotomy is revealed in the chapters dealing with immunotherapy. The

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reviews of specific and non-specific immunotherapy suggest that these approaches in animal tumour systems are still ill-defined and unproven. Yet these same approaches are being tested against a wide range of human cancers, often in conditions that would be considered unacceptable in the model animal systems. These contrasts between the experimental

animal work and the application to human cancer surely point to the need for a more critical approach to this important aspect of cancer research.

R. W. Baldwin

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Function of the cerebral cortex

The Mindful Brain: Cortical Organization and the Group-Selective Theory of Higher Brain Function. By Gerald M. Edelman and Vernon B. Mountcastle. (MIT Press: Cambridge, Massachusetts and London, 1978.)

THIS book of 100 pages consists of two papers given in June 1977 at an Intensive Study Program of F.O. Schmitt's Neurosciences Research Program (NRP). They are here published separately from the main volume of that meeting, "The Neurosciences: Fourth Study Program" (in which they will also appear), because it is thought that they are closely interrelated; and F.O. Schmitt claims in the Introduction that the two papers represent the "convergence of theory with experimentally established facts".

Vernon Mountcastle comes first with the facts. It was he who discovered that cells in the somatosensory area of the cerebral cortex were grouped according to the modality of cutaneous excitation that excited them. These groups or clusters of neurones are called columns because they run through the depth of the cortex perpendicular to its surface, and their discovery represented the first evidence for microstructure within the topographical mapping of the cutaneous surface on to the cortical surface. This chapter provides an excellent review of the anatomy of the cerebral cortex. According to Powell the basic element is a cylinder 30 μ m in diameter containing 110 neurones; Mountcastle calls these "minicolumns" and regards them as the repeating module from which the cortex is constructed. He estimates there are about 600 million minicolumns in the human cerebral cortex. They are actually smaller in diameter than the functional groups that were first described as columns in the somatosensory and visual cortex, which Mountcastle now calls "macrocolumns". According to Hubel and Wiesel's work on visual cortex there must be just under 1000 minicolumns in a macrocolumn, and just

under a million macrocolumns in the human cortex. The remarkable feature of their work on visual cortex is the high degree of regularity and order they have revealed in the arrangement of minicolumns within the macrocolumn.

It is hard to find a good account of the anatomy of the cortex, and this is a most useful review. One does not, however, emerge with much idea of what these repeating modules actually do, so one plunges eagerly into the theory that is said to converge with the facts. Gerald Edelman is best known as an advocate of the selectionist theory of the immunological response, and he here proposes a "group selectionist theory of higher brain function". It is always interesting to see what a distinguished visitor from another science makes of one's own subject. But alas it is often disappointing, and for me I fear it was so in this case. Edelman starts by postulating that "The main unit of function and selection in the higher brain is a group of cells . . .". No serious attempt is made to justify this: why not take a single cell as the unit, or a single synapse? This theoretical postulate of the group is probably the main "convergence" with Mountcastle's facts that Schmitt claims, but there is nothing at all to indicate that the group postulate is the result of a convergence; instead, it is, more likely, simply the natural starting point for someone who has been associated for many years with the NRP, and hence with the work on cortical columns. At any rate, a much better theoretical case would have to be made for postulating the group as a unit before any weight could be attached to the claimed convergence with anatomical and physiological facts.

In Edelman's theory the cell groups are selectively activated by patterns of excitation. He attaches great importance to "degeneracy", by which he means that they are excited by a range of patterns, and the range for one group overlaps that for another cell group. This is probably thought to correspond to the "partially shifted overlap" in Mountcastle's account of cortical columns. The cells of one column have some but not all properties in common with those of neigh-

bouring columns; for instance, they respond to the same modality of excitation, but come from a slightly different area of skin. At a much higher level this "degeneracy" may correspond to the fact that the units of thought and language have both specific and general attributes; a horse is a highly specific object in the sense that there are millions of other species of living things and nameable natural objects, but it is a general term in that it includes all individual horses. It is interesting to link these facts under the term "degeneracy", though it might be premature to start a search for the "horse" column in the cortex! To be serious, what seems to me to be missing in Edelman's theory is any recognition that sensory stimuli are themselves structured, and the lack of any attempt to account for the structure in the way we classify our sensory stimuli. Why pick on horses as a class of nameable objects, and for that matter why are cutaneous stimuli grouped by modality in the somatic cortex? The term degeneracy does not explain nearly enough.

My disappointment with the book as a whole stems from the fact that both authors approach the problem of higher nervous function from the same, rather narrow, viewpoint. After all, the cortex of man is responsible for his intelligence, his social behaviour, his language and, to some extent, his history. You will find little mention of the remarkable things the human brain does in these two chapters. An imaginative engineer who watches a tennis player return a service must be filled with amazement and admiration for what goes on in his brain, but these authors show no such respect for their subject matter, though to be fair they show no signs of disrespect either. Instead, they both just blandly attempt to explain consciousness; indeed they write as if this is the main problem of higher nervous function that requires explanation. I would be accused of poking fun at them if I were to quote what they have to say on the subject, but it leaves no doubt in my mind that attempting to understand the mechanisms of tennis-playing would have been a more fruitful, less premature, objective than trying to explain consciousness.

To summarise, the book contains a first rate and valuable review of cerebral neuro-anatomy, and a theory that I hope gives more insight to others than it did to me. It is safe to say that we still have much to learn about the function of the cerebral cortex.

Horace Barlow

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